BLOOD GROUPS,

BLOOD TYPES Rh, Hr ETC

FOR

CLINICAL FORENSIC

AND

ANTHROPOLOGICAL PURPOSES

BY

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B Sc (PUNJAB) M D Ch B., D P H (L POOL) F N I

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PREFACE

The author has worked on blood groups and other blood characters in three capacities concurrently. As a Serologist to the Government of India, Burma and, later, Pakistan he has answered questions raised by the Judiciary, Magistracy, Police and Bar. As an organizer of an extensive blood transfusion service in Calcutta, including the first Blood Bank in India, he has overcome many dangers and difficulties in finding donors of blood under Indian conditions. As a Professor of Serology and Immunology in the School of Tropical Medicine, Calcutta, he has taught to the various classes in the school, as a part of the curriculum, subjects dealt with in this book. He has also been in touch with anthropological workers whom he has supplied with sera and who have worked in his laboratory from time to time. All that has been reduced to writing within the covers of the book has been explained, demonstrated and discussed over a period of 15 years, excluding the newer items.

Originality is claimed for the following major items. (1) Illustrations. Out of 15, 13 are original, 6 being drawn from models designed by the author. These models enable the students to hold in their hands subtleties like blood group specific substances and their antibodies, chromosomes undergoing a reduction division in gametes, and heterozygous or homozygous red blood cells and subjects. (2) The standards for group testing sera. Western standards do not work in India. (3) Standard pipettes for measurements. The calibrated capillary pipette provides the easiest means of measuring cells and sera for grouping and typing blood. It is made easily in the laboratory. (4) A scheme of titration of

agglutinating sera (5) Classification of safe universal donors (6) Cross matching of sera (7) Establishment of several racial blood characters in the Calcutta population, including the Rh character (8) Technique of determining blood groups from stains The technique is free from the faults of old techniques (9) The simplest method of taking blood for transfusion For this purpose the use of Potain's aspirator which is available in every hospital in India, was described by the author over 12 years ago (10) Introduction of a needle into a vein Contrary to the usual teaching and natural impulse, the aperture in the needle should look downwards when the needle is introduced pointing towards the heart

In addition to the major items many minor items of special utility under tropical conditions will also be found in the book, such as preventing evaporation from preparations, and sending blood by air for grouping, Rh grouping, typing and Rh typing They are also original observations

Many convenient and expressive terms like iso(hæmagglutino)gens, iso(hæmaggluti)nins, spontaneous agglutination, centripetal agglutination, centrifugal agglutination, etc, have been introduced They have stood the test of time

The author corresponded with the late Dr G L Taylor in England during the period of extension of blood transfusion service all over the world in the late thirties, and later with Dr A S Wiener in America and *Dr R R Race in England, since the discovery of the* Rh character He is indebted to them for clarification of several points and supply of standard sera Dr Wiener has supplied him with reprints of all his articles for several years

Lengthy introductions to the innumerable items in the book have been cut out by using headings and

sub-headings in appropriate types which differentiate between the genus, species and variety at a glance, thus

Chapter headings are in **THICK CAPITALS**

Sections of Chapters are in *ITALICISED CAPITALS*

Major items in **thick type** are in the middle of the page and contain extra **CAPITAL** letters

Minor items in **thick type** begin a paragraph They may be divided further by 1, 2, 3,

Headings of illustrations and of small isolated descriptions are in THIN CAPITALS

Some items are given in *ADDITIONAL NOTES* Thus is separated the essential from the non-essential, for the primary grasp of the subject Details have not been allowed to obscure principles It is these details which, when given with the first descriptions of the principles, make a class yawn at this stage they appear unnecessary, boring and even irrelevant The APPENDIXES also separate the essential from the non-essential for the primary grasp

All the necessary references will be found in the text, in brackets They have not been allowed to interfere with the flow of thought When described as episodes they cut across the main description When given as footnotes they imitate Chinese script, necessitating reading from top to bottom Besides, the reader of an essentially technical book desires to know, in the first instance details of some rather complicated subjects, not their propounders who might as well be kept in brackets with their addresses All benefactors have been acknowledged thus at least once From nearly all of them, however, the author has derived his information on more than one occasion

Mathematically inclined readers may be disappointed at not finding in this book any top-heavy and lop-sided

equation overloaded with roots, indices and Greek letters This is due to the fact that the established data on M and N characters have been upset by the Rh character and that on the latter no stable superstructures of figures and foreign letters can be built as long as its components are not beyond dispute Besides, the author as a biologist does not do very deep obeisance to mathematics Biology defies mathematical entanglements and thrives on variation Mendel in a life time could not get his 3 1 ratio more exact than 2 8 1 2 8 1 is, of course, 3 1 and must, in fact, be 3 1, as is proved by the movements of the chromosomes in conjugation To the author, Wiener's thesis on Rh appears satisfactory on the whole A few basic equations concerning it may be included in the next edition, when more families have been studied, inheritance of Rh components in the offspring tabulated and further changes in the nomenclature stopped

History of blood transfusion has been excluded from the text and is recorded here to satisfy the historian and the critic (1) Transfusion of the blood of three boys into Pope Innocent VIII, in 1492 It was probably not a transfusion at all but a draught, in keeping with the custom of the period In an earlier period people in the same country used to rush into the arena to drink the blood of the dying gladiators (2) A technique of direct transfusion of blood from the artery of a young man to that of an old man through the mediation of two silver tubes fitting into each other Such a technique was described by Andreas Libavius in 1615 It is doubtful if it was ever put into operation (3) Bladder and quill used in injecting drugs Christopher Wren, the astronomer and architect, injected solutions of drugs into the veins of dogs by means of quills attached to bladders Later, similar solutions were injected into condemned criminals in London (4) The first authentic transfusion It was given to dogs in London by Richard Lower in 1665 (5) The first authentic transfusion in humans Denys

and Emmerez transfused 9 ounces of blood from the carotid artery of a lamb into the vein of a young man. Similar operations were then performed in England. The operation caused a death and was prohibited in France. In cases that did not die it could not have done much good because the patients passed urine 'as black as soot'. (6) No further attempts for a period of 150 years. The gap is significant. The danger was recognized universally. (7) Revival of transfusion by James Blundell in 1818. This was undertaken as a measure against hæmorrhage in midwifery practice. The interest was revived in spite of the crudeness of the apparatus. The coagulability of the blood could be guarded against, more or less, but not the incompatibility which was not understood. The incompatibility in England could not do as much harm as in India, most English subjects being either A or O unlike Indian subjects among whom the four groups are distributed more evenly. *This concludes what may well be called 'early history' of blood transfusion.* (8) The cause of incompatibility. It lay mainly in the blood groups which were described by Landsteiner in 1900–1902. The first three groups were put on a firm immunological basis by Landsteiner himself in 1900 and the fourth, the rarest group AB, was discovered by his pupils DeCastello and Sturli. Mention must also be made of Jansky and Moss who also classified bloods by their interactions, without giving reasons for the reactions, in 1907 and 1910 respectively. Only Moss's classification was adopted generally. The O A B (usually described as A O B) system although discovered before those of Jansky (I—Universal donor II, III, IV—Universal recipient) and Moss (I—Universal recipient, II, III, IV—Universal donor) did not come into general use until later, and is in this book given as 'New nomenclature' for this reason. As a matter of fact it is an old nomenclature revived because of its superiority over the other nomenclatures. (9) Plasma and serum. Their value was recognized in

World War II (10) Rh and other isohaemagglutinogens *Interest in them is very recent and shows the* importance of cross matching of blood The discovery was accidental Rbc of the brown monkey (*Macacus rhesus*) were injected into rabbits with a view to preparing an anti M serum (11) Plasma substitutes They have a bright future in intravenous alimentation

The table of contents, following the preface, gives all the items discussed in the book and will be found to be more useful than the index

Full headings of the author's papers (on which the book is based and which may be helpful to research workers in the country) are given at the end, in Appendix VI In these papers will be found references to other workers

S D S GREVAL

Calcutta,

January 1951

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I

STRUCTURES AND SUBSTANCES IN BLOOD

A droplet of fresh blood under the microscope — Mostly yellow discs, red blood corpuscles (or red blood cells), rbc hereafter (singular and plural alike), are seen Larger, irregular in shape, refractile but colourless white blood corpuscles (or white blood cells), wbc hereafter (also singular and plural alike), are also seen so are very small, round, refractile and colourless bodies, blood platelets

Constitution of blood —

Blood = FORMED STRUCTURES (rbc, wbc and platelets) + PLASMA

Plasma = FIBRIN + SERUM (separating on clotting)

Serum = SERUM ALBUMEN + SERUM GLOBULIN + metabolic products + salts + water

Serum globulin = PSEUDOGLOBULIN + EUGLOBULIN

Recently serum globulin has been discussed as

- α globulin
- β globulin, corresponding roughly to pseudo globulin
- γ globulin, corresponding roughly to euglobulin
- τ component (of β and γ) It is developed as a result of immunization with toxins

A division into pseudoglobulin and euglobulin will suffice as long as it is remembered that fractions not covered by the two divisions exist and that there is an overlap

Coagulation (clotting) of blood—Blood drawn from the body and left in a vessel which it can wet clots, COAGULATES The COAGULUM (clot) consists of semi solid plasma enclosing in its meshes the rbc, etc The plasma turns semi solid as a result of the FIBRINOGEN turning into fibrin From the clot after some time exudes the serum, a straw coloured liquid In the fluid plasma the fibrinogen remains in solution as such Recently the words plasma and serum have been used synonymously but erroneously

Rbc washed free of plasma—When the whole blood is taken into an equal or a greater volume of an isotonic solution of sodium chloride, 0 85 per cent, NORMAL SALINE hereafter, containing 1 5 per cent sodium citrate, clotting of the blood is prevented The test tube containing the mixture is then centrifuged (for about 6 to 7 minutes) The rbc fall as a deposit to the bottom of the tube leaving the slightly straw coloured plasma, diluted with citrated saline, supernatant The plasma is poured off The deposit is re suspended in saline (sodium citrate not necessary), about equal to the quantity poured off, and the tube centrifuged again The deposit re forms This is the first washing As a rule the rbc for tests and experiments are washed three times

Plasma and rbc are about equal in volume in the whole blood in man

Action of foreign plasma or serum on rbc—Generally plasma (or serum) of an animal of one species clumps together, AGGLUTINATES, the rbc of an animal of another species In the rbc there is a substance, an ANTIGEN, the HÆM(AGGLUTIN)OGEN, which is acted upon by a naturally occurring anti substance, an ANTIBODY, the HÆM(AGGLUT)ININ, in the plasma or serum The action makes the surface of the rbc sticky with the result that the rbc stick together in clumps This is HÆMAGGLUTINATION (The

term hæmogen used in this connection is different from the one which may be used in pharmacology, as 'veins' in plants are different from structures of the same name in animals)

Action of plasma or serum of the same species on rbc.—This is ISOHÆMAGGLUTINATION which in man is brought about by —

(I) Two ISO(HÆMAGGLUTINO)GENS, A and B, present in the rbc and two ISO (HÆMAGGLUTI)NINS, a and b (usually called α and β for no reason at all), present in the plasma or serum A containing rbc are agglutinated by a and B containing rbc are agglutinated by b Some subjects' rbc contain neither A nor B, while others contain both A and B The four combinations compatible with *life are the four groups*

(II) Hæmogens M and N, possibly

(III) Rh hæmogens

(IV) Other hæmogens, P, etc

(V) Cold agglutinins

Action of plasma or serum of the subject himself—Under certain conditions AUTOAGGLUTINATION occurs

The five items under the isoagglutination and the autoagglutination will be dealt with in the following pages together with their clinical, medicolegal and anthropological applications

II

THE FOUR BLOOD GROUPS

Figure 1 represents the four possibilities, compatible with life, of the distribution of the isogens and the isonins in the human blood

THE FOUR GROUPS
(O A B System)

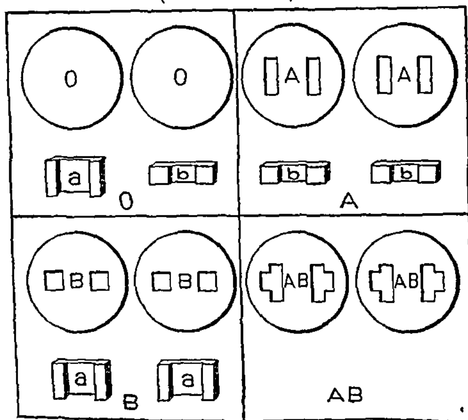


Figure 1 The discs represent the rbc O A B and AB In O there are no receptors (= no isogen) In A there are rectangular receptors (= isogen A) In B there are square receptors (= isogen B) In AB there are both receptors (= isogen A + isogen B) Specially cut pieces of wood a and b with uprights rectangular or square in cross section represent isonins a and b fitting and fastening A and B respectively Either a or b will fit and fasten AB The isogen and its fitting isonin do not occur together otherwise the fastening (= hæm agglutination) of the discs (= rbc) will occur (From wooden models)

Isogen nil, O, in rbc and isonins a and b in plasma = Group O, written O or Oab

Isogen A in rbc and isonin b in plasma = Group A, written A or Ab

Isogen B in rbc and isonin a in plasma = Group B, written B or Ba

Isogens A and B in rbc and isonin nil, o, in plasma = Group AB, written AB or ABo

The group is known by the isogen present When the expected isonin is absent, the group named by the isogen is DEFECTIVE, *e g* Oa instead of Oab

Figure 2 gives the same four possibilities

THE FOUR GROUPS
(O A B System)

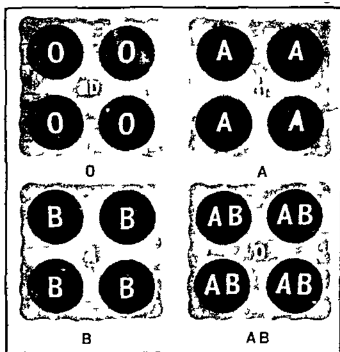


Figure 2 A and B are the isogens in the rbc and a and b are the corresponding isonins in the plasma A-containing rbc are agglutinated by a and B-containing rbc by b

The four groups indicate the only four possibilities compatible with life in which the isogens and the isonins can co-exist in the same subject

OLD EQUIVALENTS OF THE MODERN DESIGNATIONS OF BLOOD GROUPS

New nomenclature	Old nomenclature	
	Jansky	Moss
O	I	IV
A	II	II
B	III	III
AB	IV	I

The old equivalents should now be forgotten and will not be mentioned further in this account.

Other convenient styles of describing rbc, subjects and sera.—Rbc of group O, A, B and AB are rbcO, rbcA, rbcB and rbcAB respectively. Subjects of group O, A, B and AB are subjects O, subjects A, subjects B and subjects AB respectively. Suspensions of rbc O, A, B and AB are suspensions O, suspensions A, suspensions B and suspensions AB respectively. Sera of subjects O, A, B and AB are sera ab, sera b, sera a and sera o—small letter—respectively.

Serum O, serum A, serum B and serum AB (quite frequently so named) are serum ab, serum b, serum a and serum o respectively.

As the rbc are known by the isogens the serum should be known by the isonin.

Other antibodies in the plasma.—In addition to the isonins the plasma contains ISO(HÆMO)LYSINS. These are closely associated with the isonins and affect the same rbc as the latter. That is to say, the isolysin a is associated with isonin a in the plasma of a subject B, and affects the rbc of a subject A. The effect produced is one of SENSITIZATION, making the rbc sensitive to the action of the COMPLEMENT (not agglutination).

The rbc are then (hæmo)lysed by the complement present in the fresh serum This is ISO(HÆMO)LYSIS The complement occurs naturally in fresh sera and *completes* the action of sensitizing antibodies

ANTI(ISOHÆMO)LYSINS capable of destroying the lysins have been described A complete table of the corpuscular antigens and their antibodies would be

Content	The group			
	Group O	Group A	Group B	Group AB
Isogen	O	A	B	AB
Isonin	ab	b	a	o
Isolysin	ab	b	a	o
Antilysin (anti iso-hæmolysin)	(*nil*)	anti a	anti b	anti a and anti b

The antilysins do not really exist

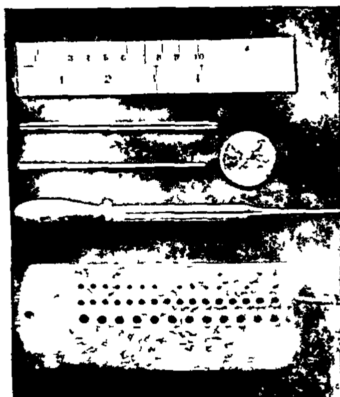
Other tissues containing isogens —The isogens are not confined to the rbc They occur in all the cells of the body as integral constituents and also in the plasma or serum as minute particles (which neutralize the isonins and isolysins and are responsible for the conception of antilysin) They are absent from the saliva and semen of some subjects who are termed NON SECRETORS opposed to the rest who are termed SECRETORS

DETERMINATION OF THE GROUP OF BLOOD

Special apparatus, chemicals, etc , used in hæmagglutination (see plates I and II) 1 Moist chambers — They are made from medium sized Petri dishes by enclosing in each set a watch glass containing a folded filter paper dripping with water The watch glass may be secured in place near the rim by plasticine A wet open preparation on a slide in such a chamber does not dry up

2 Microscope slides —Old slides which have been in use for a year or so for other procedures are preferable to new ones on which drops of rbc suspension do not

Plate I



From above downwards —

A measure indicating size

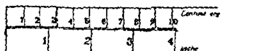
A *stirring rod with rounded end*

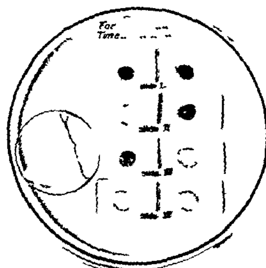
A calibrated capillary pipette lying opposite a disc with the hole no 58 of the wire gauge

A calibrated teat pipette

The wire gauge of L S Starrett & Co U.S.A. A pipette with its point fitting hole no 58 delivers 50 drops of saline to 1 cc

PLATE II







From above downwards —

A mea ure indicating ize

A moi t chamber made from Petri dishes The slides I II III and IV show hæmagglutinations or the lack of it (see text for significance) After For on the dotted line write Cells or Serum After Time on the dotted line write the time of preparing the slides In the moi t chamber is a watchglass containing a folded and wet filter paper

A hand len

spread evenly. When two or more tests are done on the same slide the obverse is partitioned by lines drawn with a glass marking pencil. Distinctive marking or writing is put on the reverse *and along the lower border* of the slide (to decide at a glance later, if the slide is lying with the right border up). The writing on the reverse although executed from under the slide should not be laterally inverted when seen from above. Four slides (and a watch glass) can be managed with perfect ease in a moist chamber.

3. Calibrated capillary teat pipettes and finger pipettes.—Their points fit the hole no. 58 of the wire gauge of L. S. Starrett and Co., U.S.A., and deliver 50 drops to 1 cc. (A single disc of hard and stainless steel with the same hole can be obtained from the Mathematical Instrument Department of the Survey of India, Calcutta. The hole widens with use and should be compared with the one in an unused disc from time to time. The same remarks apply to the gauge.)

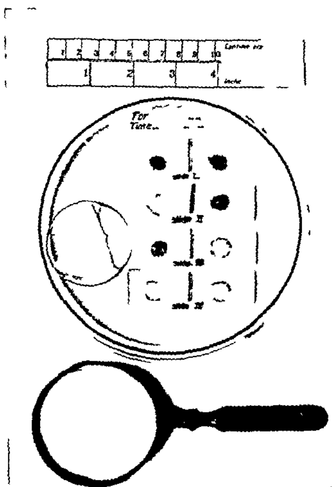
The finger pipettes are only the stems of the teat pipettes.

The pipettes in suitable containers are sterilized by autoclaving. (The teat pipettes—without the teats—are wrapped individually in brown paper and then put in a metal cylinder plugged with cotton wool. The finger pipettes are held unwrapped in a large test tube with cotton wool at the bottom.) With a suitable tilt a finger pipette picks up many drops of fluid. The movement of the rising fluid is helped by *opening and closing rapidly* the upper end with the finger. Drops are delivered with the pipette upright.

For measuring drops of whole blood (mostly for field work) the finger pipettes are dipped in 3 per cent sodium citrate solution which is allowed to dry inside. They are then sterilized in the same way.

Some pipettes are prepared with a cotton wool plug at the upper end (not projecting beyond the end). With

PLATE II



From above downwards —

A measure indicating size

A moist chamber made from Petri dishes The slides I II III and IV show hæmagglutinations or the lack of it (*see* text for significance) After For on the dotted line write Cells or Serum After Time on the dotted line write the time of preparing the slides In the moist chamber is a watchglass containing a folded and wet filter paper

A hand lens

The testing sera are inactivated at 56°C for 1 hour (the usual inactivation for 30 minutes is not sufficient)

The sera are used undiluted

[This standard for the testing sera a and b was described by the author many years ago It is preferable to the standard of a high titre—1/256, National Institute of Health, U S A, E L De Gowin and others, 1949, BLOOD TRANSFUSION, W B Saunders Company, Philadelphia and London—for two reasons (1) High titre sera are difficult to come by in India (2) A high titre is not always associated with a quick and sharp reaction

The American standard given above makes a concession for the sub group thus

Cells	Titre
A_1	1/256
A	1/128
A B	1/64

Another American standard (R B H Gradwohl 1948, CLINICAL LABORATORY METHODS AND DIAGNOSIS, The C V Mosby Co St Louis) is the following

Cells	Titre
A_1	1/40
A	1/20
A_1B	1/20
A B	1/10
B	1/40

The use of serum ab is obviously helpful for detecting O when the testing sera are weak]

2 Known rbc A and known rbc B—The rbc should react readily and well All samples of rbc A are not satisfactory for this purpose (*vide infra*, 'slow' A and A) The blood is taken in a test tube containing an equal or a greater volume of citrated saline and washed three times Several samples are needed

these, fluids are taken from sterile stocks preserved in the refrigerator

It is a pity that these pipettes are not in common use Nearly all the errors, difficulties, differences of opinions and biases in hæmagglutination are due to the difficulties of quick, accurate and repeatable measurements such as the pipettes ensure

The pipettes, after use, can be dropped into a tall cylinder containing sand or glass wool at the bottom and cleaned for further use The point will need inspection

4 **Glass rods with rounded ends** —For mixing rbc with fluids on a slide these rods are indispensable

5 **Cleaning of glassware** —It comprises (1) Preliminary wash in running water (2) Submersion in 10 per cent hydrochloric acid for a night in a glazed jar The fluid in the jar, after the glassware has been in it, should effervesce on a stone The acid digests all remains of blood The glassware should be crystal clean on inspection (3) Submersion in running water for an hour The water at the end should be tested for acid reaction (4) Submersion in distilled water (5) Change of distilled water The water should be tested with silver nitrate for remains of tap water (6) Jerking free of water (7) Drying on racks (8) Drying in oven (9) Sterilizing in autoclave or oven (without cotton wool plug most pieces with plugs certain pieces only) Sand and glass wool are cleaned in the same way

Testing sera and testing rbc 1 Known serum a (from subject B) and known serum b (from subject A) —They should in a dilution of 1 in 5 agglutinate known rbc A and known rbc B almost immediately and should not affect known rbc O (*vide infra*, anti O serum) If such potent sera are not available a serum ab (from subject O) should also be included isonins a and b in a serum ab are on the whole stronger than those occurring individually

Examine immediately and then every 15 minutes for ½ hour (without removing the slide from the moist chamber) Record result at the end of ½ hour under each compartment, drawn on a piece of paper according to the following notation

Rbc agglutinated in clumps which have moved to the periphery of the preparation (agglutination centrifugal)	++
Rbc agglutinated in clumps which have remained where they formed or have moved to the centre on rotating the slide (agglutination centripetal)	+
Intermediate	+ (±)
Granularity only	±
Suspension as it was	—
++ clumps sometimes become large plaques and move centrally They are best recorded as	++
± is not likely to be encountered with potent sera	

If only a agglutinates the unknown rbc are A
" b " " B
If neither a nor b agglutinates, the unknown rbc are O
If both a and b agglutinate the unknown rbc are AB

A suspension agglutinated by serum ab is not one of rbc O even if the indifferent testing sera appear to leave it untouched

In plate II, slides I, II, III and IV indicate groups O, A, B and AB respectively

The known rbc from different subjects will be used for the next step

2 **For unknown serum**—Add undiluted unknown serum to the 2 per cent suspensions of the known rbc, thus

Known suspension A + 1 drop	Unknown serum 1 drop	Known suspension B + 1 drop	Unknown serum 1 drop

Mix, spread, watch and read as before

If the serum agglutinates suspension A only it is a
" " " B " b
" " both A and B ab
" " neither A nor B o*

*Serum o comes from group AB

From blood taken for other purposes (*e g* the Wassermann reaction), rbc can be washed out of the divided clot in saline, if the clot is fresh and in a good state of preservation The coarse particles are rejected after they have settled down and the suspension washed twice

From the final deposit make a 2 per cent suspension thus (1) Measure and deliver into a tube 1 cc normal saline with an ordinary graduated pipette (2) Remove from the tube 1 drop with a calibrated test pipette (3) Add to the tube 1 drop of washed rbc deposit with the same test pipette (4) Shake to make a homogeneous suspension (1 drop in 50 drops = 2 drops in 100 drops = 2 per cent) The rbc will become ' known ' after the test

Setting up tests 1 For unknown rbc—Make a 2 per cent suspension and add to the known sera, thus

With potent sera

Known a	+ Unknown suspension	Known b	+ Unknown suspension
1 drop	1 drop	1 drop	1 drop

With indifferent sera

Known a	+ Unknown suspension	Known b	+ Unknown suspension	Known ab	+ Unknown suspension
1 drop	1 drop	1 drop	1 drop	1 drop	1 drop

Mix with the special rod (one end for each mixture) and spread over an area about 1 5 cm in diameter Place in moist chamber *Hold the moist chamber in both hands* and move quickly in a small circular orbit, clockwise and then counter clockwise, with an up and down tilt Impart a side to side movement off and on These movements (*i*) make the suspension of blood uniform in the first instance, (*ii*) help in hæmagglutination later, and (*iii*) aid the eye in the end, the agglutinated masses of rbc becoming easier of detection on movement

donors, 2nd quality', if their sera just agglutinate rbc A and rbc B in 1 in 16 but not in 1 in 50 No more than 200 cc of blood may be transfused into an adult from a safe universal donor of 2nd quality Other 'universal donors' are 'dangerous' *Rbc O will not suit some recipients A, and AB or even B* (see p 17)

An older standard is that a 1 in 9 dilution of the donor's serum should not agglutinate an equal volume of the recipient's rbc in a 50 per cent suspension

MAKING OF DILUTIONS OF SERA FOR TESTING SAFETY OF DONORS

1 One in 50 dilution Measure into a test-tube 1 cc of saline Remove 1 standard drop Add 1 standard drop of the serum under test and shake Use 2 drops for testing Discard 2 drops, leaving 46 drops in the tube

2 One in 16 dilution To the balance left in the tube add 2 standard drops of serum Ignoring the small quantity of the serum removed previously there will now be 3 drops of serum in 48 drops of the total fluid This is, for all practical purposes, equal to a 1 in 16 dilution

3 One in 9 dilution For performing the single and simple test needing the dilution accuracy is employed To 8 standard drops of saline add 1 standard drop of serum

Universal recipient—Blood of subjects AB having no isonins in it cannot agglutinate rbc of any donor hence the term 'universal recipient' for a subject AB But he can be killed by donors of all other groups with strong enough isonins For safety, donors of all other groups for recipients AB should satisfy the same conditions as the universal donor for other groups

In plate II, if the slides were prepared with known rbc for identifying the unknown sera, slides I, II, III and IV would indicate sera from groups AB, B, A and O respectively

The rbc and serum from the subject under examination must agree When they do not agree an explanation must be found (*vide infra*)

A ± reaction or even an unexpected — reaction is possible Double the dose of the serum and observe again If the expected reaction of an isonin is not seen the isonin is absent and the group is defective The author, however, has not yet found a defective group An extremely weak reaction is likely to turn negative if the serum is weakened by an extra drop of saline as has been advised by most previous workers The extra drop of saline is supposed to guard against the action of cold agglutinins (*vide infra*) It is not necessary

A weak serum may also fail to act with an rbc suspension which is considerably stronger than 2 per cent

Theoretically a zone phenomenon is possible an undiluted serum not agglutinating the rbc may do so on dilution The author has never seen such an occurrence

The technique so far described employs slides only The rbc and sera can be tested in small test tubes also, as is done routinely for Rh incompatibility (*vide infra*) For determining the group of blood there is no advantage in using tubes

Universal donor —Rbc O having no affinity (no receptor) for either of the two isonins cannot be agglutinated by any recipient hence the term universal donor for a subject But the isonins in a donor O, if strong enough, can kill recipients of all other groups The author terms O donors ' safe universal donors, 1st quality ', if their sera do not agglutinate 2 per cent rbc A and rbc B in a 1 in 16 initial dilution, and ' safe universal

OTHER UNUSUAL REACTIONS

These reactions are (1) Intra group reaction in group B (2) Agglutination of rbc O by sera other than a_2 (3) Agglutination caused by serum o (from subject AB)

The following table gives all the unusual reactions (other than those occurring in group B) and their bases

Group (including sub group)	Isonins occurring normally	Isonins occurring abnormally
A	b (β)	Anti-O also called a_2 (α_2) very rare Reacts with all O rbc and 95 per cent A_1 rbc
A_2	b (β)	a_1 (α_1) (1-2 per cent) Reacts with A_1 and A_1B rbc
A_1B	None	Anti-O Same as in A_1
A_2B	None	a_1 (α_1) Same as in A_2 but occurs much more frequently than in A_2 (25-30 per cent)
B	a (α)	Anti-O very rare The same as in A_1 and AB
O	a (α) and b (β)	None (On the contrary a or b is said to be absent at time The group is then defective The author has not come across such a group)

The importance of cross agglutination for the purpose of transfusion of blood is obvious

Intra group reactions in group B await study The group being rare in Europe and America has not been studied so well as groups O and A

COLD AGGLUTININS

Isonins against otherwise compatible rbc exist but are operative in the cold only At body temperature they are inert *but may be activated to act at room temperature by a previous transfusion of specially susceptible rbc*

THE SUB GROUPS

Isogen A is divisible into A_1 and A (A described a few years ago has not survived) The four groups thus become six —

O, A_1, A , B, A_1B and A B

A and A B are less frequent than A_1 and A_1B

Determination of A —Take 2 cc serum α (from subject B) and divide into 2 equal parts To one part add an equal volume of a deposit of washed rbc suspected to be A (by slowness and/or weakness of reaction), shake into suspension, and leave at room temperature ½ hour, in the cold ½ hour and at room temperature again ½ hour Centrifuge to remove the supernatant fluid, absorbed serum α It will not agglutinate another sample of the same (suspected rbc), yet it will agglutinate ordinary (unsuspected) rbc A, *if the suspicion is well founded* The suspected A is A Any other sample of rbc A reacting with the unabsorbed serum but not with the absorbed serum will also be A

The absorbed serum is serum $α_1$ (or serum anti A_1)

(A , if suspected, can be differentiated in the same way from A_1 and A)

UNUSUAL REACTIONS ASSOCIATED WITH SUB GROUPS

Sera of some subjects A_1 and A_1B —They are known to agglutinate rbc A , rbc A B and rbc O They are a sera or anti O sera Anti O sera is a better description in as much as such sera agglutinate all rbc O without exception

Sera of some subjects A and A B —Conversely to the last occurrence they agglutinate rbc A_1 and rbc A_1B

The deposit is examined best over the concave mirror of a microscope lying on its side (*see* Rh)

BACTERIOGENIC AGGLUTINATION

Old rbc agglutinate on the addition of human serum, independent of the isonins An enzyme produced by bacteria acts on the rbc and activates a latent antigen T Anti T agglutinins are present normally in all human sera hence the agglutination Bacterial agglutination is also called pan agglutination

PSEUDO AGGLUTINATION

This semblance of iso agglutination is produced by three causes (1) Rouleaux formation due to the action of some sera, including the sera of some recipients Under the microscope rouleaux can be seen in the clumps There is no difficulty in examining the preparations of the author's technique under a high power dry lens The slide is removed from the moist chamber, examined and returned without touching the rbc A coverslip is not necessary (2) Wharton's jelly in the umbilical cord blood sera When the slide is moved the rbc form clumps which break up when the slide is at rest This phenomenon has been called FALSE AGGLUTINATION in as much as it does not last The same effect can be seen with anti M and anti N fluids and occasionally in titrating sera a and b, towards their end points (D Harley, MEDICOLEGAL BLOOD GROUP DETERMINATION, 1944, London, William Heinemann Medical Books, Ltd) The term is hardly appropriate under or alongside Pseudo agglutination FLEETING AGGLUTINATION would be more suitable (3) Coagulation of plasma in unwashed or inadequately washed rbc In anæmic states recipient's unwashed or inadequately washed rbc sometimes cause this complication The coagulation can be distinguished from agglutination

AUTO AGGLUTININS

They are probably cold agglutinins which become active due to an abnormal interference by a transfusion or to a morbid process At times they may become as strong as to render impossible, at room temperature, the counting of rbc in spite of the dilution of blood, which occurs in the hæmocytometer pipette

Recently cold agglutinins and auto agglutinins have been demonstrated in the blood of cases of Primary Atypical Pneumonia (J C Turner *et al*, 1943, *Lancet*, *i*, 765) and used as a means of diagnosis Patient's own rbc or rbc O are used

The technique of diagnosing Primary Atypical Pneumonia—The blood of the patient is incubated at 37 C and the serum is removed at the same temperature The tests are set up at room temperature The serum can be stored in a refrigerator but must be tested within a week, as the storage lowers the titre

In small Wassermann tubes are made four serial dilutions of the serum 1 in 8, 1 in 16, 1 in 32 and 1 in 64 Each dilution is mixed with an equal volume of a 1 per cent suspension of patient's own rbc or rbc O in another set of tubes The tubes are shaken and left overnight at 2 to 4°C Readings are made while the tubes are still cold Only macroscopical reading is accepted A titre of 1 in 32 or over is accepted as diagnostic

(The titre is given by the *initial* dilution not the ultimate dilution)

The phenomenon of true cold agglutination disappears when the tubes are incubated at 37 C This step will guard against the abnormal anti O isonins in some subjects The hemagglutination due to isonins commences at room temperature, before the tubes are put in the refrigerator, and becomes apparent on the rbc settling down Such an agglutination will not disappear at 37°C

The deposit is examined best over the concave mirror of a microscope lying on its side (see Rh)

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SPONTANEOUS AGGLUTINATION

Well washed rbc, preserved in plugged tubes in a refrigerator, are occasionally seen to show agglutination This corresponds to what is also seen occasionally in bacterial suspensions The phenomenon is due to a change in the rbc apart from the activation of the latent antigen T Washing does not improve the rbc The merest suspicion of agglutination renders the rbc unfit for any test This auto agglutination is different from the one caused by the auto agglutinins mentioned above

CROSS MATCHING FOR TRANSFUSION OF BLOOD

When the donor and the recipient belong to the same group —

(i) For rbc and sera

Donor's rbc 2% 1 drop	+	Recipient's serum 1 drop	Recipient's rbc 2% 1 drop	+	Donor's serum 1 drop

There should be no hæmagglutination as a rule As an exception, intra group hæmagglutination occurs, because of (1) The abnormal isonins occurring in sub groups of A The reaction between 2 subjects A should be watched with particular care, especially after a previous transfusion of blood (2) Rh incompatibility (*see* Rh) (3) Type incompatibility (*see* M N) (4) Pregnancy some cases (5) Morbid state in the recipient (6) Iso immunization with other hæmogens after a previous transfusion (*see* OTHER HÆMOGENS, P, Le, Fy, etc)

The single step of testing the rbc of the donor with the serum of the recipient has been called MAJOR CROSS MATCHING The other step of testing the rbc of the recipient with the serum of the donor has been called MINOR CROSS MATCHING its utility in

excluding dangerous donors from causes other than group incompatibility is obvious

For second and subsequent transfusions in males and even first transfusion in married females (for reasons connected with pregnancy) Rh incompatibility is specially important The reaction with the recipient's serum should also be tested in a small test tube (W R tube), thus (1) Mix recipient's serum, 3 standard drops, with 2 per cent suspension of donor's rbc, 3 standard drops (2) Incubate at blood heat 1 hour (3) Examine the deposit over the concave mirror of a microscope lying on its side (*see* Rh for details and yet another test)

(*a*) *For sera only*

Donor's serum 1 drop + Recipient's serum 1 drop (1)	Donor's serum 2 drops (2)	Recipient's serum 2 drops (3)

There should be no increase in opalescence in (1) unless an increase is seen in (3) also, due to change in the *recipient's serum on exposure*, (2) *is a control of a* double volume of serum for comparing appearances It should not become opalescent on exposure

The increase in opalescence in (1) only will be due to causes other than those falling under iso hæmagglutination, other immunological reactions which must be avoided For this purpose a scratch test may also be done on the recipient with the donor's serum On the upper arm are made 2 scratches, $\frac{1}{4}$ inch long and 1 inch apart, without drawing blood The donor's serum is rubbed into the upper scratch and normal saline into the lower The result is read immediately, in 15 minutes and in 1 hour There should be no swelling suggestive of urticaria with the serum If time permits a complement fixation test between the two sera may also be undertaken It should be negative (Recipient's serum, 1 in 5, 1 vol + donor's serum, 1 in 5, 1 vol + guinea pig complement 2 HMD in 1 vol room temperature $\frac{1}{2}$ hour and

incubation ½ hour + sensitized sheep rbc 1 vol incubation ½ hour = complete hæmolysis Serum controls will be necessary Recipient's serum control consists of the same reagents excepting the donor's serum in place of which normal saline is used Donor's serum control consists of the same reagents excepting the recipient's serum in place of which normal saline is used Recipient's serum may be anti complementary in which case an allowance for more complement must be made The donor's serum must not be anti complementary)

When a universal donor is used—Only the rbc of the donor will not (ordinarily) be agglutinated by the serum of the recipient The opposite combination is bound to produce hæmagglutination (isonins a and b + rbc of any group other than O = hæmagglutination)

For second and subsequent transfusions in males and even first transfusion in certain females guard against Rh incompatibility of the rbc of the donor and use tubes also as before

When a universal donor is being used for a recipient O, it is not being used as a universal donor but as a donor O

When a donor other than AB is used for a universal recipient—Only the serum of the recipient will not (ordinarily) agglutinate the rbc of the donor The opposite combination is bound to produce hæmagglutination (Isogens AB + isonins a and/or b from any group other than AB = hæmagglutination) The serum of the donor, therefore, must satisfy the standards laid down for a safe universal donor (*vide supra*) Guard against Rh incompatibility as before

DETERMINATION OF BLOOD GROUPS FROM STAINS

The basis of the process is the absorption of the known isonins by the unknown isogens of the stains

proved to be caused by human blood only (*see* Appendix, Precipitin Reaction)

Special apparatus—In addition to the usual apparatus, etc, used in grouping blood the following are required (1) Conical tubes about 7 cm long and 5 mm in diameter, made from glass tubing, in the laboratory (2) Corks to fit They are obtained from suppliers of glassware, etc, to perfumers (3) A stout platinum wire mounted on a handle (4) Plasticine (5) A wire cage for holding moist chambers used for holding the conical tubes stuck sub horizontally with plasticine (6) A bag for holding the cage (7) A personal seal for sealing the bag This is necessary because the work is usually undertaken for medicolegal purposes

Selection of a suitable serum ab—A potent serum ab in which titre of a equals that of b is selected and its Minimal Dose of Equal and Simultaneous Agglutination, MDLSA, determined, thus

(*a*) A preliminary 3 slide test is carried out as follows —

Suspension rbc A 1 vol + Serum ab 1 vol	Suspension rbc B 1 vol + Serum ab 1 vol

Suspension rbc A 1 vol + Serum ab 2 vol	Suspension rbc B 1 vol + Serum ab 2 vol

Suspension rbc A 2 vol + Serum ab 1 vol	Suspension rbc B 2 vol + Serum ab 1 vol

The agglutination should be equal and simultaneous Otherwise the serum is rejected

(*b*) The minimal dose of equal and simultaneous agglutination is determined next by dilution of the serum as follows —

(*i*) With the standard capillary pipette add 1 drop of the serum to 1 cc of saline and draw a vertical line on the tube This is a 1 in 50 dilution (approx) Test two standard drops If agglutination occurs in 2 minutes and is simultaneous and equal the dilution is further diluted to 1 in 100 (approx) by adding 1 cc of saline to the same tube The new dilution is tested as before Observations last ½ hour

The author has no experience of a weaker dilution being effective

(*ii*) If agglutination does not occur in a 1 in 50 dilution, one more drop of serum is added to the dilution and recorded on the tube by another vertical line The balance of 1 in 50 dilution consisted of 49 drops For all practical purposes the first drop of serum added to it was still in it Another drop added means 2 drops of the serum in a total of 50 drops or a 1 in 25 dilution The dilution is tested as before

(*iii*) If no agglutination occurs in a 1 in 25 dilution, one more drop of serum, the third drop, is added to the dilution in the tube, which measures 48 drops For all practical purposes the 2 drops of serum added previously are still in it The dilution now is 3 in 49 or 1 in 16 (approx) This dilution is tested as before

(*iv*) If no agglutination occurs in a 1 in 16 dilution, *one more drop of serum, the fourth drop, is added to the* dilution which measures 47 drops For all practical purposes the three previously added drops are still in it The dilution now is 4 in 48 or 1 in 12

(*v*) In the same way further additions of drops are made and stronger dilutions obtained The outgoing drops, for testing are looked upon as drops of saline

only, the small quantity of serum in them being ignored The dilutions corresponding to

1		50	
2		25	
3		16	
4		12	
5		9	
6	drops are 1 in	8	respectively
7		7	
8		6	
9		5	
10		4	
11		4	
12		3	

For counting drops every 4 vertical lines are crossed by a fifth oblique line

The dilutions are really slightly weaker than those shown

The advantage of this method of titration is that although 13 dilutions (including the 1 in 100 dilution, not shown in the parallel lines of figures) are tested from each serum only one tube is needed Four sera can be tested side by side in one Petri dish Limitation of space and the corresponding concentration in observation result

The weakest dilution causing an equal and simultaneous agglutination is the MDESA

The simultaneity and equality may cease to exist at any dilution of the serum The MDESA cannot then be determined

Absorption of the known isonin —25 milligrams of the stained fabric (cut into strips 1 2 mm wide) or 10 milligrams of scraped blood are put into a conical tube and 0 1 cc of the serum dilution containing 3 MDESA in a unit volume is added The contents are mixed well with the stout platinum wire and the tube corked The corked tube is fixed sub horizontally in a moist chamber

and left for half an hour at blood heat and overnight at ice box temperature The moist chamber for this purpose is put in a wire cage which is enclosed in a bag sealed with personal seal Usually there are several moist chambers employed on one occasion

(The serum dilution containing 3 MDESA in a unit volume is made thus If a 1 in 16 dilution of the serum agglutinates an equal volume of 2 per cent rbc A and rbc B, the MDESA is 1 in 16 A dilution containing 3 MDESA will be 3 in 16 It will be observed that the *dose* is expressed by the *strength* of the dilution as the *volume* remains *constant* D is an abbreviation for 'dose' or 'doses' alike)

MDESA may be replaced by MDSA provided the equality is perfect in a preliminary 3 slide test The titration to determine the minimal dose is carried out as before The two sides should show a distinct and simultaneous agglutination which may not be equal The dose giving such a result is MDSA

Two kinds of controls are set up (1) Controls of stains of known blood groups The stains should be as old or older than those under test They should give the expected reaction (2) Controls of the unstained material corresponding to the stained material Of surfaces (wood, plaster, etc), an equal unstained area is scraped to the same depth as the stained area has been scraped From surfaces which cannot be scraped (glass, china, metal, etc), an equal area is marked off by a circle drawn with a match dipped in melted hard paraffin The area is washed in 3 or 4 drops of saline dropped on it and sucked back by a pipette The washings are dried It should be noted, of course, if the stain is on a clean surface or super imposed on a surface already stained by some other material a control of this material will also be necessary if the stain is superimposed A fabric which must not be cut up is also washed like a surface after it has been stiffened by paraffin applied to the reverse

The tubes are removed from the ice box next morning. Their contents are packed tight with the stout platinum wire (unless they are of the nature of saw dust which does not stay packed). The wire is washed between two packings. The tubes are centrifuged. A clear slightly coloured fluid becomes available mostly on a deposit and occasionally under a scum. Drops are removed for testing with calibrated capillary finger pipettes (one for each tube). Packing to centrifuging takes half an hour. If not, extra time is allowed at room temperature to eliminate the activity of any cold agglutinins present.

The clear fluid must be tinged, otherwise the specimen must be regarded as insoluble and therefore unsuitable for the test.

Test after absorption.—The absorbed serum dilution is tested for loss of isonins with 2 per cent known rbc A and rbc B. (i) If rbc A are not agglutinated the stain has absorbed the isonin a, the blood in the stain, therefore, was of group A. (ii) If rbc B are not agglutinated the blood in the stain was of group B. (iii) If neither rbc A nor rbc B are agglutinated the blood in the stain was of group AB. (iv) If both rbc A and rbc B are agglutinated the group of the blood in the stain was O. The double hæmagglutination should occur in 5 minutes. An agglutination occurring later is ignored. Characteristically the reaction occurs well within 5 minutes.

Three additional lots of controls are also set up. (1) Controls of the rbc A and B used. There should be no spontaneous agglutination. (2) Controls of 3 MD(E)SA with suspensions of rbc A and rbc B. The degree of hæmagglutination should be of a high order. (3) Controls of 1 MD(F)SA with suspensions of rbc A and rbc B. The degree of hæmagglutination should be good. The control is specially important when working with MDSA only. The end result in the test proper should be like that of the control in equality.

and left for half an hour at blood heat and overnight at ice box temperature The moist chamber for this purpose is put in a wire cage which is enclosed in a bag sealed with personal seal Usually there are several moist chambers employed on one occasion

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necessary The isogens differ in their capacity to absorb the isonins so much as to make accuracy in weight unimportant Further, excessive drying interferes with the solubility of the stain

The weights given in this account for stains and scraped blood have been found to be suitable by trial and error

The weight is kept constant for the stained fabric A fine fabric, area for area, weighs less than a coarse one and also holds less blood in its meshes A coarse fabric weighs more than a fine one and also holds more blood

The stains in medicolegal work in India are mostly encountered on cotton fabric

Absence of colour in the serum dilution after contact with the stain—The rejection of the specimen is advisable for medicolegal purpose, in spite of the fact that some workers have determined the blood groups from tissues from mummies

Preservation of the testing serum—The serum will keep in a refrigerator for several weeks if phenolized (0 35 per cent) It can also be freeze dried (without phenolization) The MDESA, however, must be confirmed with new lots of abc

Retardation of agglutination (1) When the serum after absorption agglutinates both suspensions—The reaction is only interpreted when the speed of the agglutination, its equality and its intensity are identical with those of the serum control containing 3 MD(E)SA Such a reaction is given by the serum after absorption with stains of group O so characteristically that the author feels justified in ruling out the identification of group O in the absence of the reaction

Some sort of semblance of equality of agglutination of the two suspensions may be found after a contact of 30 or even 15 minutes with a serum ab which has been

(The abc are collected with special care on the day of the titration of the serum ab and preserved in two sets of plugged test tubes in the refrigerator. One set is used for the test on the following day, and the other may be needed for a repetition of the test.)

The specimens from exhibits are examined after the controls have given the expected results. Only clear cut agglutinations unaccompanied by ± reactions are accepted. Otherwise 'the reaction is such that no opinion can be given.' Dilution of the absorbed fluid may abolish the ± reaction. It is done as follows —

To the balance of the fluid in the conical tube (from which 2 drops have been removed for testing) are added 3 standard drops of saline. As the total fluid measured 5 drops (= 0.1 cc), the balance also consists of 3 drops and is diluted to half its original strength, namely, ½ MD(E)SA.

The mixing, packing and centrifuging are done again. In all probability the excess of serum giving the ± reaction will be eliminated (= made ineffective) by dilution.

If the dilution reduces the previously + reaction on the other side also, again 'the reaction is such that no opinion can be given.'

Considerable failure to obtain results is acknowledged by workers in America. In India, the failure is even greater, probably due to more sweat and animal matter, which absorb isonins, in clothes.

Only the four blood groups can be determined satisfactorily from the stains.

Fecal contamination destroys the isogens.

Additional Notes on the Technique

Weight of the dried blood in a stain.—Accurate weighment of the dried blood contained in a stain is not

Advantages of the absorption test over the extraction and demonstration of the isonins from stains — The isonins are not found in the extract in a satisfactory titre The author failed to demonstrate them in most stains The titre of the isonins in India is low on the whole

The group of the blood in the stain may be 'defective' The isonins which can co exist in the group compatibly with the isogens may be lacking It must, however, be added that in over 2,000 cases grouped for clinical purposes, in connection with the local blood transfusion service, in the Imperial Serologist's Laboratory in Calcutta, a defective group was never found

In experimental work occasionally the author failed to obtain reactions of the desired distinctiveness from known stains, that is, he obtained negative results He, however, never obtained false results This feature of the test depends on the fact that in the technique all borderline reactions are eliminated by dilutions and then excluded from consideration

In testing the actual exhibits failures to obtain results are very frequent Firstly, some of the controls from the unstained material interfere with the isonins, equally or unequally the specimens corresponding to the controls are then not proceeded with Secondly, in the reaction of some of the specimens proceeded with a lack of distinctiveness occurs these specimens are discarded Thirdly, the main specimen with which other specimens in a case are to be compared is sometimes among those which have been discarded the other specimens are not then proceeded with

Rejection of a stain when the control (unstained material) has shown absorption, partial or complete, of one or both isonins —Other workers have shown that in a surprisingly large number of cases the unstained material will contain one or more of the blood group receptors, presumably from sweat, urine, animal material,

absorbed with other groups also. It is due to incomplete absorption. Reaction of group O, therefore, must be read within 5 minutes.

(2) When the serum after absorption agglutinates one suspension only.—More often than not the rbc will not be agglutinated with the same speed and intensity as are seen in the case of the stronger of the two serum controls. The agglutination will begin more slowly and will usually be frank, not sharp. With the absorption of the appropriate isonin some loss of the non-appropriate isonin also occurs. According to other workers this loss is either non-specific or indicative of a partial binding together of the two isonins. The author adds the observation that, at least in an evenly balanced serum ab, the loss does not depend on the serum but on the stain. In a batch of absorption experiments with the same serum some A and B stains will absorb non-specifically b and a respectively to some extent, others will not. That is why frank agglutinations are insisted on. In such agglutinations the non-specific process has stayed in the background.

(3) When the serum after absorption does not agglutinate either suspension frankly.—A semblance of double agglutination may be present as a ± agglutination in both the suspensions. Agglutination on one side may even become almost frank after some time. On dilution the frank reaction disappears. On such a reaction opinion is withheld. That it has not resulted from contact of the serum with stain of group O is evidenced by the delay in appearance of the reaction and the lack of intensity and equality of agglutination. It may be caused by absorption with a weak isogen combined with *a non-specific absorption of the non-appropriate isonin*. It may also be caused by absorption with isogens AB (of group AB) both of which are weak and one is weaker than the other.

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Rejection of a stain when the control (unstained material) has shown absorption, partial or complete, of one or both isonins—Other workers have shown that in a surprisingly large number of cases the unstained material will contain one or more of the blood group receptors, presumably from sweat, urine, animal material

etc, and consequently a positive test for this receptor in the stained portion would mean nothing If only one receptor is found in the unstained material, tests, it has been recommended, may be carried out to detect the possible presence of the other, though it will readily be appreciated that in such a case we cannot expect to establish with certainty the full and exact group of the stain The author adds the observation that the comparative concentration of the sweat, etc, in the stained and the unstained material cannot be judged The stain bearing area may have in it more sweat, etc, than the unstained area and may show, consequently, more absorption Deductions from a comparative absorption in this case will be misleading The very existence of such a possibility, if explained fully, will create in the minds of the jury a reasonable doubt as to the interpretation of the test He, therefore, rejects all specimens the controls of which have absorbed the isonins

Technique for smaller quantities—The quantities of the stain and the serum dilution used in the absorption test are 25 mg of the former and 5 drops, from a specially standardized pipette, of the latter But 20 mg and 4 drops or 15 mg and 3 drops will suffice, provided that pipettes with thinner ends are used in removing the fluid from the tube after absorption and in adding drops of the suspensions Dilution will be difficult

Similarly, quantities of the dried blood scraped from exhibits and the serum dilution can be reduced from 10 mg of the former and 5 drops of the latter to 8 mg, 6 mg or even 4 mg of the former and 4 drops, 3 drops or even 2 drops of the latter

Very small stains are treated by washing with 5 to 6 drops of the serum dilution containing 2 MD(E)SA in a volume The dilution is held in contact with the stains in a teat pipette By squeezing out and sucking back the dilution a reasonably coloured and turbid fluid is

soon obtained The fluid is incubated for half an hour at 37°C and removed to a refrigerator for the night Next morning it is treated like the ordinary specimen

To check the spread of the dilution from the stains melted (but not very hot) hard paraffin may be used as in washing large stains

The author has obtained indications of groups from signatures on paper, in blood, of a certain fraternity A positive report for forensic purposes, however, could not be given on mere indications

A macro-technique versus a micro-technique—The author prefers the former to the latter Manipulation of slides repetitions of observation and discrimination between a mere sedimentation of rbc and agglutination are all easier in the macro than in the micro-technique Further, experience in microscopy is not needed the results can be read even by a jury, if necessary

Special features of the technique—There are two such features (1) The MDESA or MDSA The sera providing this dose do not give false results due to heterologous absorption Such a result has been reported (D Harley, 1936, *Brit Jour Exper Path*, **17**, 35) (2) The use of 3 MDESA or MDSA This constant dose keeps the appearance of the hæmagglutination in the test constant on all working days, more being used of a weak serum and less of a strong one The author prefers strong sera

DETERMINATION OF GROUP FROM STAINS OF HUMAN ORIGIN OTHER THAN BLOOD STAINS

Absorption with known isonins will also demonstrate the isogens A and B in the stains of saliva, nasal secretion and semen provided the subjects concerned are *secretors* (secrete the isogens in their saliva) Only a seminal stain can be identified as such If such a stain absorbs one or both isonins the group of the subject is indicated

as usual In the case of non absorption, however, the subject may either be non secretor or belong to group O

Certain secretions may be presumed to be present on certain objects, e g saliva on cigarette ends (*see* chapter on forensic medicine)

ADDITIONAL NOTES ON ISOGENS AND ISONINS

Substances A and B —They can be prepared, for the purpose of absorbing isonins a and b, from the salivas of secretors of subjects A and B Clear saliva, free from bubbles, is collected from an angle of the mouth in a test tube It is heated at 100 C, in a water bath, for 10 minutes to destroy the enzymes and most of the micro organisms found in the mouth It is used the same day or left in a refrigerator

The substances are also prepared from other sources and are available commercially

Isonin a.—Certain lots of anti sheep rabbit hæmolytic amboceptor (amboceptors made in rabbits against sheep rbc for complement fixation) act like isonin a They can be used in the place of serum a Obviously in the rbc A there is present an antigen related to the Forssman antigen present in sheep rbc

Natural sera a and b and immune sera anti-A and anti B —Some early workers on blood groups worked with antisera made in rabbits against known rbc A and rbc B All that work is vitiated by the fact that in addition to the substances A and B there are found in the human rbc substances M and N, Rh of several types and possibly many other hæmagglutinogens

Isogen and Hæmogen —The author makes a distinction between the terms The hæmagglutinogens A and B he calls isogens because they produce iso hæmagglutination normally The other hæmagglutinogens he calls hæmogens

Substance O —O is not merely a negation of A and B but an active antigen in its own right The anti O sera

(present abnormally in sub groups A_1 and A_1B) react with it. It is held that these sera agglutinate rbc A_2 because of the presence of O in them, their genotype being A_2O (*see* GENETICS OF BLOOD GROUPS, BLOOD TYPES, RHESUS GROUPS, ETC.). O is also an isogen.

Blood character C.—There is evidence for the existence of this new character. It is associated with A_1 and B but not with A_2 and O. The reactions of the four isogens with five antisera are given below:—

Isogen	Reaction with sera				
	Anti A	Anti A	Anti B	Anti C	Anti O
A_1	+	+	−	+	−
B	−	−	+	+	−
A_2	−	+	−	−	+
O	−	−	−	−	+

Serum anti A_1 is the serum a absorbed with rbc A.
Serum anti B is serum b.
Serum anti C is the antiserum against the new blood character.
Serum anti O is serum a occurring abnormally (*see* page 17). (Adapted from A. S. Wiener, 1949, *Bulletin of the New York Academy of Medicine, second series*, **25**, 255–260.)

Starting a blood transfusion service without standard testing sera and rbc.—Sera and rbc from 6 volunteers are tested and agglutination obtained thus:

Sera of volunteers 1–6	Rbc of volunteer 1–6					
	1	2	3	4	5	6
1	−	+	+	+	+	−
2	−	−	+		−	−
3	−	+	−	−	+	−
4	−	+	−	−	+	−
5	−	−		+	−	−
6	−	+	+	+	+	−

as usual In the case of non absorption, however, the subject may either be non secretor or belong to group O

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2	−	−	+	−	−	−
3	−	+	−	−	+	−
4	−	+	−	−	+	−
5	−	−		+	−	−
6	−	+	+	+	+	−

The bloods fall into three batches —

Batch one It consists of inagglutinable rbc in columns 1 and 6 Volunteers 1 and 6 are therefore subjects O Their sera confirm the findings by agglutinating the rbc of all other volunteers

(Group AB is not represented no serum is without action on all rbc)

Batch two It consists of columns 2 and 5 because of similarity of reactions of the rbc and sera

Batch three It consists of columns 3 and 4 because of the similarity of reactions of the rbc and sera

It will be observed (i) that the sera of subjects in batch two act on the rbc of subjects in batch three and (ii) that the sera of the subjects in batch three act on the rbc of subjects in batch two The batches two and three are, therefore, composed of subjects A and B respectively or B and A respectively with their sera a series of more subjects is then tested The serum reacting with the rbc of the greater number is likely to be derived from a subject A, as group B predominates in India In testing a series from European community the serum reacting with the greater number is likely to be derived from a subject B, as group A predominates in Europe The rbc of the subject A can also be agglutinated by the anti sheep rabbit hæmolytic amboceptor

Strictly speaking the question of obtaining the sera by trial and error should not arise at all The worker must know his own group It is possible, in most cases, to obtain all that is necessary to start work from one subject (1) Subject A provides serum b (2) Subject B provides serum a The sera a and b, even if not up to the standard will help in grouping other subjects who may provide better sera (3) Subject O often has isonins a and b of unequal potency The stronger of the two must be known (4) Subject AB often has isogens of unequal potency The stronger of the two must be

known The worker, before starting a small laboratory, will, of course, carry out all the necessary tests on his own blood in a well established laboratory where inequalities in rbc A will be accounted for and several samples of rbc and sera of different groups will be available Only subjects O with equally potent isonins and subjects AB with equally potent isogens will be at a disadvantage The subject may find in his fresh serum isolysin which will aid in detecting its appropriate isogen Only one isolysin may be found in the serum of a subject O

Besides the isogens and the isonins the worker will also know all about his other hæmogens, M N, Rh, etc

Preservation of testing sera.— A serum taken with aseptic precautions will keep in a refrigerator (4–8°C) for many days Some testing serum is always kept frozen in the freezing compartment of the refrigerator When required, it is thawed slowly, in the refrigerator, overnight Means can be devised to freeze dry small quantities of sera with an air pump and liquid oxygen Sera so dried keep good for months and can be sent to other localities by post

Preservatives and dyes are also used Phenol diluted with ether can be added to quantities as small as 1 cc (see anti M and anti N fluids) Preservatives which colour the two sera differently are used thus (1) For serum b, from group A To each cc add 0 01 cc each of 1 per cent aqueous solution of neutral acriflavine and 0 5 per cent solution of basic fuchsin (2) For serum a, from group B To each cc add 0 01 cc of 1 per cent aqueous solution of brilliant green

The author has not found it necessary to colour the sera

Prevention of pseudo agglutination of rbc— Formation of rouleaux to which the pseudo agglutination

The bloods fall into three batches —

Batch one It consists of inagglutinable rbc in columns 1 and 6 *Volunteers 1 and 6 are therefore* subjects O Their sera confirm the findings by agglutinating the rbc of all other volunteers

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Subject to these considerations there is no harm in using a group testing serum without inactivation by heat. The complement in human serum is not very strong and disappears in a few days of storage in the cold (ordinary refrigerator temperature outside the freezing chamber). This is inactivation by aging. The human anti Rh sera are not inactivated by heat (see Rh).

Effect of heat and light on isogens.—The isogens exposed to heat and light become insoluble and inactive by denaturation. The specimens of known stains should be preserved in a cool, dry and dark place. For washings from stains in medicolegal work see the section on forensic application of blood characters.

Hapten.—An antigen which is so modified by denaturing that it ceases to be a full antigen is a hapten. It can absorb the antibody formed by the full antigen, but cannot give rise to one.

Wbc and platelets.—They do not appear to be affected by isonins. It has not been considered worth while to test them in cross matching blood for transfusion.

is due does not occur when the rbc are suspended in a solution of lecithin The solution is made thus (i) mark a test tube for 20 cc , (ii) dissolve in the tube 0 5 gm of lecithin in 10 cc of ether, (iii) add 20 cc normal saline, (iv) heat in water bath until the solution is free from the smell of ether and below the 20 cc mark, (v) cool quickly under a cold water tap, (vi) add distilled water up to 20 cc mark (vii) filter the opalescent solution through paper

In lecithin solution the rbc become spherical but retain their specific agglutinability

The author has not found it necessary to use lecithin solution The rouleaux can be seen under the microscope and distinguished from clumps of agglutination They should be so distinguished routinely in cross matching blood

Preservation of rbc—*See* M N system

Complement in sera—If the testing serum is not inactivated by heat, the complement may hæmolyse the rbc under test Even when no colour resulting from the hæmolysis is discernible, the complement often lowers the titre of the isonins probably by interfering with the surface of the rbc through an otherwise inappreciable lytic action

When the serum is inactivated for 30 minutes only, in the 3 slide test for selecting an evenly balanced serum ab (for determining blood groups from stain by absorption) it sometimes happens that the hæmagglutination *on the second slide (with 2 volumes of serum)* is poorer than on the first slide (with 1 volume of serum) A trace of colour is also discernible usually This paradoxical effect disappears on inactivating the serum for 1 hour

The zone phenomenon in hæmagglutination, if it occurs, is probably due to the interference by the complement

III

THE THREE TYPES

M and N Hæmogens

The type depends on the hæmogens which are quite unrelated to the isogens A and B and occurs as M, N or MN in all subjects of all groups, thus

THE THREE BLOOD TYPES

(M N System)

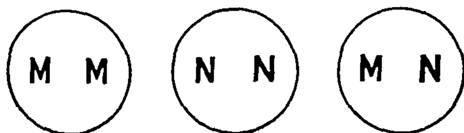


Figure 3 Hæmagglutinogens M and N are present in 3 combinations

No subject, unlike the subject O in the O A B system, is free from M and N They differentiate three types in each group and thus make possible 12 descriptions of blood A blood may be

OM	ON	OMN
AM	AN	AMN
BM	BN	BMN
ABM	ABN	ABMN

If the two sub groups A and A B are included, 6 more descriptions, 18 in all, become possible

The hæmogens M and N have also been called cryptically and unnecessarily M and N 'factors'

Unlike what occurs in O A B system, no antibodies corresponding to the hæmogens are present normally in

(This is an old scheme which gave good results The scheme detailed under Rh for the preparation of the anti Rh serum may also be tried)

Ten days after the last injection the animals are bled The serum is distributed in lots of 1 25 cc in small phials which are corked and sealed with paraffin,' inactivated, and stored frozen (in the freezing chamber of a refrigerator)

Absorption of anti-OM and anti ON sera with O and obtaining of the residual anti-M and anti-N fluids

(1) Absorption of anti-OM serum —The writer has found that a good serum when diluted 1 in 25 (two drops of serum from the calibrated pipette, added to 1 cc of saline from which two similar drops have been removed make a 2 in 50 dilution, addition of an equal volume of saline gives a 1 in 50 dilution, higher dilutions of 1 in 100 and 1 in 200 are obtained, if desired, by the same process of doubling the volume) agglutinates both the OM and ON in an equal volume of a 2 per cent suspension in 30 minutes The clumps of the OM rbc appear more compact in comparison with those of the ON cells None of his sera has agglutinated the O rbc in a 1 in 50 dilution

One cc of the serum (or a little over, as much as can be removed from the phial) is added to 1 cc of a packed deposit of ON cells in a 15 cc centrifuge tube and the two mixed by gentle tapping and rotation The rbc are agglutinated into a gelatinous mass The tube is left in an almost horizontal position in a moist chamber, for half an hour at room temperature, for half an hour in a refrigerator and for half an hour again at room temperature It is then centrifuged and a clear fluid (with or without a tinge of colour) measuring 0 1 cc to 0 2 cc more than the added serum (due to a tighter packing of the rbc) separated

One drop of the absorbed fluid is tested with one drop of a 2 per cent OM rbc If agglutination com-

the human blood Only a few abnormal cases have been reported The antibodies may, however, be formed after a transfusion

Testing Fluids

They are the anti M fluid and the anti N fluid which are prepared in rabbits from known OM and ON bloods

Collection of OM and ON bloods —OM and ON bloods from professional donors are collected in Rous and Turner's solution, as recommended by Wiener (A S) as follows 3 8 per cent sodium citrate solution, in lots of 20 cc, is sterilized in 100 cc flasks, 5 4 per cent glucose, in lots of 50 cc, is sterilized in 250 cc flasks, 30 cc of blood from a donor are drawn, added to the citrate solution, and the whole added to the glucose solution The mixture is kept in a refrigerator About 15 cc of a packed deposit of rbc can be obtained from 100 cc of this mixture Required quantities can be withdrawn with a 10 cc pipette Strict sterility must be maintained The mixture remains fit for use for over two months A slight or even marked colour in the supernatant fluid can be ignored and so may be darkening of the deposit as long as there are no smell, turbidity or other signs of bacterial growth

The mixture is centrifuged and the supernatant citrate and glucose solution removed The deposit is washed once in sterile saline These operations are done in rubber capped centrifuge tubes when the rbc are required for injection For absorption the capping of the tube is not necessary

***Injection of rabbits for anti OM and anti ON sera* and collection of the sera** —Full grown rabbits weighing about 3 lb (the usual weight of a rabbit purchased in Calcutta) are injected intravenously with a 50 per cent suspension of the rbc as follows —

1st injection	1 cc
2nd injection on the 4th day	1 cc
3rd injection on the 8th day	2 cc
4th injection on the 12th day	4 cc

(This is an old scheme which gave good results. The scheme detailed under Rh for the preparation of the anti Rh serum may also be tried.)

Ten days after the last injection the animals are bled. The serum is distributed in lots of 1.25 cc in small phials which are corked and sealed with paraffin,' inactivated, and stored frozen (in the freezing chamber of a refrigerator).

Absorption of anti-OM and anti-ON sera with O and obtaining of the residual anti-M and anti-N fluids

(1) Absorption of anti-OM serum.—The writer has found that a good serum when diluted 1 in 25 (two drops of serum, from the calibrated pipette, added to 1 cc of saline from which two similar drops have been removed, make a 2 in 50 dilution: addition of an equal volume of saline gives a 1 in 50 dilution; higher dilutions of 1 in 100 and 1 in 200 are obtained, if desired, by the same process of doubling the volume) agglutinates both rbc OM and ON in an equal volume of a 2 per cent suspension in 30 minutes. The clumps of the OM rbc appear more compact in comparison with those of the ON cells. None of his sera has agglutinated the O rbc in a 1 in 50 dilution.

One cc of the serum (or a little over, as much as can be removed from the phial) is added to 1 cc of a packed deposit of ON cells in a 15 cc centrifuge tube and the two mixed by gentle tapping and rotation. The rbc are agglutinated into a gelatinous mass. The tube is left in an almost horizontal position in a moist chamber, for half an hour at room temperature, for half an hour in a refrigerator and for half an hour again at room temperature. It is then centrifuged and a clear fluid (with or without a tinge of colour) measuring 0.1 cc to 0.2 cc more than the added serum (due to a tighter packing of the rbc) separated.

One drop of the absorbed fluid is tested with one drop of a 2 per cent OM rbc. If agglutination com-

mences in about two minutes and is complete in five minutes the potency is regarded as good

One, two, and three drops of the absorbed fluid are next tested, on a slide marked into three compartments with one drop of 2 per cent ON rbc If no agglutination occurs in any of the compartments in 30 minutes the specificity of the fluid is regarded as good

If agglutination (definite or even a granularity) occurs with one, two or three drops, the specificity is defective The serum must be reabsorbed with 1 cc to 0 5 cc (depending upon the degree of the agglutination) of packed ON rbc The reabsorption does not lower the titre for OM rbc

The absorbed fluid of a good potency and specificity is the anti M fluid It is collected with a capillary teat pipette, transferred to a phial, and preserved with 0 35 per cent tricresol For the purpose of adding tricresol the volume of anti fluid is looked upon as 1 cc One drop of the following mixture added to 1 cc of anti fluid gives the desired 0 35 per cent

Dilute 0 2 cc of tricresol with 0 6 cc ether In the total volume of 0 8 cc the dilution is 1 in 4 Discard 0 1 cc *of the mixture To the remaining 0 7 cc add* 0 3 cc more of ether

The mixture is based on the following calculation

50 droplets in 100 cc	= 1 per cent
17.5 100 cc	= 0 35 per cent (0.35 × 50 = 17.5)
Of tricresol diluted 4 fold	
70 droplets in 100 cc	= 0.35 per cent (17 5 × 4 = 70)
Or 0 7 droplets in 1 cc	= 0.35 per cent
Of 0 7 cc increased to 1 cc	
1 droplet in 1 cc	= 0.35 per cent

As the major part of the droplet consists of ether which ultimately leaves the serum a deduction for its bulk from the total is unnecessary

One cc of the fluid suffices for 40 to 50 tests

(2) Absorption of anti ON serum—*The serum* when diluted 1 in 100 to 1 in 200 agglutinates both rbc

OM and ON in an equal volume of a 2 per cent suspension The ON rbc are made more compact in comparison with the OM cells

The serum is diluted 1 in 4 to 1 in 8 with normal saline *The rest of the procedure is the same as described* for the absorption of the anti OM serum, with OM substituted for ON

The reabsorption of the anti ON serum with OM rbc lowers the titre for ON rbc also The finished anti N fluid, however, acts as briskly as the anti-M fluid, the reason being that a 1 in 4 dilution of a serum found agglutinating perfectly in a 1 in 100 dilution and giving a ± reaction in the next higher dilution is stronger than the pure serum which agglutinates in a 1 in 25 dilution but not in a 1 in 50 dilution The discrepancy is due to the inequality of spacing in the geometrical series, 1/25, 1/50 1/100, 1/200

Such was the position before the discovery of Rh Now the rbc used for injection into the rabbit and those used for absorption should have the same *Rh* content (*see* Rh) or those for injection should be Rh —

The situation has recently become even more complicated for 2 reasons (1) Variants of the hæmogens M and N have been found They are of the same order as the variants of the isogen A but appear to be rare (2) A new hæmogen S has been discovered It is associated with M and N and thus creates another set of variants in the hæmogens (A S Wiener, 1949, *Bulletin of the New York Academy of Medicine loc cit*) The hæmogens with the variation are M, Ms, N and Ns The selection of the OM and ON rbc for immunizing the rabbits is no longer an easy procedure

The Technique of Typing

The procedure is similar to the one followed in grouping a suspension of rbc with known sera, a and b

In the left hand compartment of the slide use a drop of anti M fluid and in the right hand compartment a drop of anti N fluid Add a drop of the unknown suspension, mix and leave in the moist chamber

If only anti M fluid agglutinates the type is		M
anti N		N
both fluids agglutinate		MN

Read the results in 5 minutes, 15 minutes and 30 minutes After 15 minutes no new change occurs A suspension giving a ± reaction in 5 minutes and not progressing further is retested with 2 drops of the fluid and a + reaction obtained A + reaction is obtained in most cases in the first instance

[For differentiating between M, Ms, N and Ns, 3 anti fluids are used thus

Serial number	Reaction with fluids			Hæmogens present (or type)
	Anti M	Anti N	Anti S	
1	+	−	−	M
2	+	−	+	Ms
3	−	+	−	N
4	−	+	+	Ns
5	+	+	−	MN
6	+	+	+	MNs

The hæmogen S (or the anti S fluid) is not available freely yet]

The Life of Rbc

It was believed to be of the order of 30–40 days (C L Evans, 1925 RECENT ADVANCES IN PHYSIOLOGY, J & A Churchill Ltd, London) A determination by direct observation was made possible by the discovery of M N M + rbc transfused into an M − recipient could be detected for over 100 days (P Levine 1935, *Jour Lab & Clin Med* **20,** 785) These rbc were not altogether compatible because of a difference of type of the M—N system The life of a subject's own rbc must be considerably longer than 100 days

M-N in other Cells

Evidence for or against the presence of the hæmogens in other cells must be obtained afresh in view of what has been said under the preparation of the anti fluids. The presumption is that the hæmogens, like the isogens, occur in all cells of the body.

IV

THE Rh +/– STATE THE RHESUS GROUP (THE Rh FACTOR)

The Rh hæm(agglutin)ogen in the human rbc was discovered 9 years ago (A S Wiener and K Landsteiner, 1941, *Jour Exper Med*, **74**, 309) Almost immediately afterwards its relationship to erythroblastosis fœtalis was traced (P Levine, 1942, *New York State Journ Med*, **42**, 1928) It also explained why when incompatibility of groups, dangerous universal donor and even incompatibility of types were excluded, blood transfusions at times not only did not improve the blood state of but actually killed the recipient

The hæmogen is found normally in the rbc of the Indian brown monkey, *Macacus rhesus* the Rh are the first two letters of the specific name

A rabbit (or a guinea pig) injected with the rbc of the monkey develops, in its blood, an antibody against the Rh and supplies the animal serum for testing human blood In certain dilutions the serum agglutinates the rbc of a certain proportion of human subjects These subjects are Rh + Those whose rbc are not so agglutinated are Rh –

Another source of the testing serum is an Rh – mother who has carried an Rh + fœtus She has become ISO IMMUNIZED with the rbc of the fœtus, which have strayed from its circulation into hers The iso antibody formed by the mother damages the rbc of the fœtus hence the disease The fœtus may be cast off as an abortion or delivered as a diseased infant suffering from jaundice, dropsy and anæmia in various degrees Such a mother (or expectant mother) comes to grief herself if transfused with Rh + blood Those connected with the

pre Rh blood transfusion schemes and blood banks knew that unexpected accidents occurred more often in maternity cases than in other cases

In a male Rh — recipient the transfused Rh + rbc also iso immunize the patient and may be destroyed more or less speedily the case may or may not be benefited After a second transfusion from the same donor (or another Rh + donor) the male recipient too comes to grief like the Rh — mother

The figures for the Rh + and Rh — subjects vary with the race Amongst the Europeans they are of this order Rh + 85 per cent, Rh — 15 per cent Amongst Indians they became available in 1943 in Calcutta and were of this order Rh + 90 per cent, Rh — 10 per cent (S D S Greval and A B Roy Chowdhury, 1943, *Journ Ind Med Assoc*, **13,** 65) They were confirmed in Calcutta in 1944 (C R Das Gupta, 1944, *Indian Med Gaz*, **79,** 372) They were contradicted in Bombay in 1945 (V R Khanolkar and L D Sanghvi, 1945, *Nature*, **155,** 427) They were confirmed again for Asiatic Indians (by examination of sailors) in America (A S Wiener, 1945, *J Immunol*, **50,** 341) The latest negative figure for Indian students in London is 9 5 per cent (C H Prasad, E W Ikin and A E Mourant, 1949, *The American Journal of Physical Anthropology*, **7,** 553)

Dangers of the incompatibility of the Rh state are two

(1) Accidents of blood transfusion The risk in married females is higher in as much as the accident may occur in the first transfusion When only an animal serum is used in the test many reactions are ' doubtful ' (±) They are taken as positive (+) in determining the Rh state of a donor But in a recipient they should be taken as negative (—) and an Rh — donor provided (An Rh — donor can do much less harm to an Rh + recipient than an Rh + donor to an Rh — recipient) Further, a very small proportion of Rh — subjects, found

to be negative by animal testing serum, may be positive (Rh′ and Rh″, *see* Rh types) by human testing serum Special care is, therefore, necessary in cross matching the blood of married females The accidents though most likely to occur during pregnancy and soon after parturition, may occur after an abortion and long after the child bearing age The Rh — males transfused with Rh′ and Rh″ blood (which will be returned negative by animal serum) may not benefit by the first transfusion The accident, however, will occur only with the second transfusion and can be prevented by a careful cross matching of bloods Weak agglutinins can be detected by the use of anti human globulin serum (R R A Coombs, A E Mourant and R R Race, 1945, *Brit Jour Exper Path*, **26**, 255), or more simply by conglutination test (*see* Blocking antibody) Absolute safety can be guaranteed after a biological test in which a sample of the donor's blood is injected into the recipient and one hour later a sample of blood is drawn from the latter to see if the serum is tinged with hæmoglobin (A S Wiener, I J Silverman and W Aronson, 1942, *Amer Journ Clin Path*, **12**, 241, *vide infra*) If the serum is not tinged, a previously transfused male, an expectant mother or a mother can be safely transfused from the donor Safety of the fœtus, however, cannot be guaranteed when the test is undertaken for the benefit of the mother The small quantity of the incompatible blood used in the test may raise the titre of the mother's anti Rh serum and increase the damage to the fœtus The danger is less in India in proportion to the lower Rh — rate but is not negligible

(2) Erythroblastosis fœtalis (and consequent abortions and sterility) For more details *see* p 75

Intra-group and anomalous reactions unconnected with Rh hæmogen are known to be given by pregnant women Besides, anti Hr and St sera from Rh + expectant mothers have been described They agglutinate Rh —

or heterozygous Rh + rbc (A J McCall, R R Race and G L Taylor, 1944, *Brit Jour Exper Path*, **26**, 255)

PREPARATION OF AN ANIMAL TESTING SERUM

Rabbits are immunized as for the production of a hæmolytic amboceptor from the rbc of the brown monkey, according to the following plan —

1st intravenous injection of 1 cc of 50 per cent rbc
2nd intravenous injection of 1 cc of 50 per cent rbc on the 2nd day
3rd intravenous injection of 1 cc of 50 per cent rbc on the 3rd day
4th intravenous injection of 1 cc of 50 per cent rbc on the 4th day
5th intravenous injection of 1 cc of 50 per cent rbc on the 5th day

On the 5th day after the last injection a small sample of blood is collected in a capillary pipette from the ear vein The serum from the sample is inactivated in a capillary pipette at 56 C for 1 hour (½ hour does not suffice for the complete destruction of the complement as has been stated before) From it are prepared two dilutions a 1 in 500 dilution and a 1 in 1,000 dilution

One drop of each dilution (beginning with the weaker dilution) and 1 drop of a 2 per cent suspension of the monkey rbc are placed on a slide, mixed thoroughly with a glass rod with rounded ends (the end moving clockwise and then counter clockwise 7 times or more, until the suspension has become homogeneous), spread over an area of about 1 5 cm in diameter and left in a moist chamber Two dilutions can be tested on one slide

The slide is examined every 15 minutes for one hour with a hand lens

A 1 in 500 dilution should show a distinct hæmagglutination with the naked eye and a 1 in 1 000 dilution a distinct granularity (made plainer with the lens)

If the serum is not up to the mark, another course of 3 or 5 injections is started the same day (anaphylaxis

will not occur after an interval of 5 days only) The test is repeated 5 days after the last injection

If the serum is up to the mark, the animal is bled from the heart 5 days after the test (10 days after the last injection), and the serum is separated and inactivated at 56 C for one hour. The dilutions tested are 1 in 1,000, 1 in 1,500, 1 in 2,000, 1 in 2,500 and 1 in 3,000. Four slides can be left in one moist chamber. The last dilution giving an appreciable granularity is the minimal agglutinating dose, MAD, and the next dilution (without granularity) is the sub MAD. Ten sub MAD are used for a testing dose for human rbc

If, for instance, the last dilution giving an appreciable granularity, in one hour, be 1 in 1,500, the MAD is 1 in 1,500 and the sub MAD is 1 in 2,000. The testing dose for the human rbc is 1 in 200

In addition to the testing dose, a dose twice as strong is used as a supporting dose which in the instance given would be 1 in 100 or, preferably, 2 drops of the same dilution may be used, instead of 1 drop

(From a full grown rabbit in Calcutta 10 to 12 cc of blood can be taken without killing the animal which can be used again for producing the anti monkey rbc serum. The first injection after an interval of more than a week is given intraperitoneally. Then the rest follows the routine)

Whether the sub MAD is correctly selected will be shown by the reaction of the serum with known rbc (*see* **Superiority of the slide technique**)

Every serum is not suitable for testing blood. A suitable serum will give (*i*) clear cut positive and negative reactions and (*ii*) expected percentage of negative reactions

The clear serum to which is added 0 35 per cent tricresol is distributed in lots of 0 2 cc in small phials. The phials are corked and inactivated at 56°C for one

or heterozygous Rh + rbc (A J McCall, R R Race and G L Taylor, 1944, *Brit Jour Exper Path*, **26**, 255)

PREPARATION OF AN ANIMAL TESTING SERUM

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3rd intravenous injection of 1 cc of 50 per cent rbc on the 3rd day
4th intravenous injection of 1 cc of 50 per cent rbc on the 4th day
5th intravenous injection of 1 cc of 50 per cent rbc on the 5th day

On the 5th day after the last injection a small sample of blood is collected in a capillary pipette from the ear vein The serum from the sample is inactivated in a capillary pipette at 56 C for 1 hour (½ hour does not suffice for the complete destruction of the complement as has been stated before) From it are prepared two dilutions a 1 in 500 dilution and a 1 in 1,000 dilution

One drop of each dilution (beginning with the weaker dilution) and 1 drop of a 2 per cent suspension of the monkey rbc are placed on a slide, mixed thoroughly with a glass rod with rounded ends (the end moving clockwise and then counter clockwise 7 times or more, until the suspension has become homogeneous), spread over an area of about 1 5 cm in diameter and left in a moist chamber Two dilutions can be tested on one slide

The slide is examined every 15 minutes for one hour with a hand lens

A 1 in 500 dilution should show a distinct hæmagglutination with the naked eye and a 1 in 1,000 dilution a distinct granularity (made planer with the lens)

If the serum is not up to the mark another course of 3 or 5 injections is started the same day (anaphylaxis

A -- in compartment 1 makes a — in compartment 2 doubly certain hence the term supporting dose

A ± both in compartments 1 and 2, without any difference is occasionally recorded The granularity is really due to agencies other than anti Rh body Such a reaction though recorded other than — is looked upon with suspicion

The tube technique—Each suspension needs two 4 inches × ½ inch tubes, thus

	Tube for test proper	Tube for control
Dilution of the testing dose	3 drops	Nil
Same dilution of normal rabbit serum	Nil	3 drops
Suspension	3 drops	3

The tubes are shaken, left in an incubator at blood heat for one hour The deposit is then examined over the concave mirror of a microscope laid on its side and the results recorded, thus —

Agglutinated mass of rbc with irregular surface and edge	++
Mass of rbc with crinkly edge	+
Deposit of rbc with smooth edge which puts out a tongue on tilting the tube	—
Deposit of rbc with smooth edge which does not put out a tongue on tilting the tube	±

The control tube should show a smooth edge which puts out a tongue on tilting the tube

All animal sera agglutinate rbc of all newborn infants and are therefore useless in determining whether an infant is Rh + or Rh — *For this purpose the human testing serum must be used*

Superiority of the slide technique—Besides being economical the slide shows the difference in hæmagglutination much better than the tubes in the following details (1) Size of the agglutinated masses (2) Whether (*i*) centrifugal, (*ii*) centripetal, (*iii*) mixed, or (*iv*) changing from centrifugal to centripetal by forming plaques

hour They are then stored in the freezing chamber of the refrigerator For use a phial is taken out of the chamber and allowed to thaw slowly in the refrigerator overnight Dilutions made from the liquefied serum for use are kept in the refrigerator One phial lasts several days

The serum may also be put in 1 cc ampoules for despatch by post

Animal sera have been produced recently from goats on a commercial scale

TECHNIQUE OF TESTING BLOOD WITH ANIMAL TESTING SERUM

The suspension of rbc—A 2 per cent suspension is used Preferably the blood should be taken freshly *in a citrate solution Washed rbc from a fresh clot can* also be used

The slide technique—Each slide is marked into three compartments on the obverse On the reverse is given the distinctive number or mark along the lower border The left hand *compartment is for the support*ing dose, the middle compartment for the testing dose and the right hand compartment for a dilution of normal rabbit serum giving the same amount of froth as the dilution of the testing dose, thus

1	2	*3*
Supporting dose	Testing dose	Normal rabbit serum

If the testing dose of the immune serum (from immunized animal) be 1 in 200, the dilution of the normal serum will be stronger The froth is produced by *proteins, the content of which in the immune serum is* greater than in the normal serum

All quantities are measured in standard drops The dilutions are deposited first and then is added the suspension The mixing is done as usual The results are read every 15 minutes for an hour

Yet another difficulty arises in grasping the fact that the recessive character, responsible for the Rh negative state, is not really non existent somatically as the character t (d would be better) is non existent *vis a-vis* T, in the genetics of the edible pea in Mendelism (*see* chapter on genetics) With appropriate reagents both Rh and rh can be detected (D F Cappell, 1946, *Brit Med Journ*, ii, 602)

What applies to the negative character rh applies to the positive characters with greater justification no character in the genetics of Rh is recessive

Once these difficulties are recognized and thus overcome the combinations of the antigens responsible for the types, is given in the scheme of the discoverer of the Rh antigen, are easy to follow Additions made to the scheme by other workers are not necessary

Rh types made easy—The antigens are only three in number Rh_0, Rh′ and Rh′ They occur singly or in combination, thus

Serial No	Antigen 1	Antigen 2	Antigen 3	Type as named
I	Rh			Rh
II	Rh	Rh		Rh
III	Rh		Rh	Rh_2
IV	Rh	Rh	Rh″	Rh Rh
V		Rh		Rh
VI			Rh	Rh
VII		Rh	Rh	Rh Rh
VIII				rh (Rh negative)

Two combinations of antigens have been given special names (1) Serial No VII, Rh′ + Rh″, is called Rh_y and (2) Serial No IV, Rh_0+Rh′+Rh″, is called Rh

These details are used in comparing two or more lots of animal testing sera on (i) samples of strongly agglutinable rbc and (ii) samples of weakly agglutinable rbc With proper adjustment of the dose all sera should give the same results (*vide supra*, correct selection of the sub MAD) Any serum showing a difference is rejected

In the author's opinion inferior results obtained by early Rh workers on slides were due to (i) inaccurate measurements, (ii) insufficient mixing of the serum and the rbc, (iii) insufficient time usually allowed for reaction on slide, and (iv) difficulty of reading + and ± results when all the rbc have settled down more or less uniformly on the slide—the settlement will not occur if the slides are agitated every 15 minutes This applies to potent sera With weak sera tubes give better results Different doses may be taken for the tubes and slides

Rh AND Hr BLOOD TYPES

Nothing in the immunological constitution of human red blood cells has grown so luxuriantly in such a short time as the details of the Rh antigens Eight years ago there was one *antigen*, Rh, responsible for two *characters*, (i) Rh positive, Rh, dominant and (ii) Rh negative, rh, recessive To day there are three antigens and eight types In addition, there is the Hr antigen (Hr 'factor ')

The use of the indeterminate term 'factor ' for the definite terms 'antigen ' (in immunology) and 'character ' (in genetics) has not contributed to the comprehensibility of the situation

Another difficulty is the departure from the usual method of describing the basis of characters in genetics The genes carrying the characters are composite, not single One, two or three antigens (physical bases of characters) may occur together at the locus of a gene on the chromosome and are inherited together by the offspring

clinically only the antigen Rh_0 appears to be potent in this respect

The author in this book retains the original symbols, with CAPITAL R

Hr types made easy —Hr is an antigen like the other hæmogens and is found in association with Rh Like the Rh it has a composite nature and consists of Hr°, Hr′ and Hr″ Rh and Hr with the same distinguishing mark are mutually exclusive

Corresponding to the 3 antigens there are 3 antisera anti Hr°, anti Hr′ and anti Hr″ The reactions of the 8 types of Rh with 3 anti Rh sera and 3 anti Hr sera are given below

Type	REACTION WITH ANTI RH SERA			REACTION WITH ANTI HR SERA		
	Anti Rh_0	Anti Rh′	Anti Rh″	Anti Hr°	Anti Hr′	Anti Hr″
rh	−	−	−	+	+	+
Rh′	−	+	−	+	−	+
Rh″	−	−	+	+	+	−
Rh′Rh″	−	+	+	+	−	−
Rh_0	+	−	−	−	+	+
Rh_1	+	+	−	−	−	+
Rh_2	+	−	+	−	+	−
Rh_1Rh_2	+	+	+	−	−	−

(Adapted from A S Wiener 1949 *Bulletin of the New York Academy of Medicine loc cit*)

The St serum may be considered synonymous with anti Hr′ serum When the term Hr is used in a general sense, the Hr′ character is meant

All early work reported on Hr was carried out with anti Hr′ serum

The incompatibility of an Hr positive donor for an Hr negative recipient has not yet been commented upon Evidently such an incompatibility exists and provides the important anti Hr serum The use of a donor of the type rh as a universal donor, therefore, cannot be absolutely safe

The types are the allelic genes, simple or composite Some of the types are common, others rare

All the types can be determined by three antisera, Anti Rh_0, Anti Rh′ and Anti Rh″, thus

If only anti Rh agglutinates the type is Rh
anti Rh — Rh
anti Rh″ — Rh″
all 3 fail to agglutinate — ih
anti Rh and anti Rh agglutinate the type is Rh
anti Rh and anti Rh — Rh_2
anti Rh Rh and anti Rh — Rh Rh
anti Rh and anti Rh — Rh Rh

Rh_1 is the commonest type

It will be seen that four of the types contain the antigen Rh_0 while only one of them is known as the type Rh_0

It will also be seen that four types are without the antigen Rh_0

Recently three types have been designated thus

Type Rh is to be rh
Rh — rh
Rh Rh″ — rh rh

Moreover, all types denoted by small r (rh, rh , rh″ and rh rh) are to be called Rh negative

The eight types now are

Rh, Rh, Rh, Rh Rh — Rh positive (They are detected as positive without differentiation between the antigens by an animal testing serum They contain Rh_0)

rh, rh″, rh rh″, rh — Rh negative (They are detected as negative without differentiation between the antigens by an animal testing serum They do not contain Rh)

A recipient falling under any of the four negative types cannot be transfused with blood from a donor falling under the positive types, because of the antigen Rh_0 All such recipients should be transfused from a strictly Rh negative donor of the type rh if not from a donor containing the same antigens as themselves

The possibility of the antigens rh′ and rh sensitizing recipients not possessing them remains, although

Rh-Hr system—It is postulated that the three related Rh factors have three additional reciprocally related Hr factors (A S Wiener, 1949, ABRAHAM LEVINSON ANNIVERSARY VOLUME, STUDIES ON PEDIATRICS AND MEDICAL HISTORY, Froben Press Inc, New York) This concept unifies the Rh Hr system like the CDE system

CDE SYSTEM

This is Fisher's system which postulates 3 characters in a composite gene at a locus (quoted by R R Race, 1946, *Brit Med Bulletin*, **4**, 188) It includes both Rh and Hr characters

Stated simply, in the interest of the primary grasp of the nomenclature, C = Rh′, D = Rh_0, and E = Rh″

The three anti Rh sera are known as anti Rh or anti C, anti Rh_0 or anti D and anti Rh″ or anti E *The C of the CDE system is entirely different from the C associated with the O A-B system*

Though originally not designed for this purpose these equivalents have been used by some American workers in remedying the lack of short (phenotypic, *see* GENETICS OF BLOOD GROUPS, ETC) names in the Fisher system (De Gowen, Harden and Alsever, *loc cit*) Rh_1, the commonest type, for example is called CDe, because the rbc of the subject have reacted with anti C serum and anti D serum but not with anti E serum

According to this plan the equivalents of the Rh types in the CDE system are as follows

Rh positive	Rh_0	= cDe	D positive
	Rh_1	= CDe	
	Rh_2	= cDE	
	Rh_1Rh_2	= CDE	
Rh negative	Rh′	= Cde	D negative
	Rh″	= cdE	
	Rh′Rh″	= CdE	
	rh	= cde	

THE EIGHT Rh BLOOD TYPES

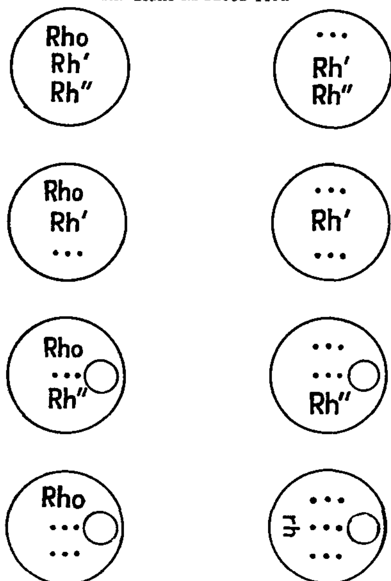


Figure 4

The rbc contain the various antigens in 8 combinations Divided equally and vertically 4 combinations on the left are Rh positive and 4 combinations on the right are Rh negative (3 from top clinically and the last at the bottom also serologically) Divided equally and horizontally the lower 4 combinations have an additional constituent This is Hr Originally when Hr' represented the entire Hr only these combinations were taken to be Hr positive

precision Such an accomplishment, however, is not possible in biology which is based on variation and defies precision attained through mathematical subtleties

Rh VARIANTS

Variants of Rh′ antigen—Rbc of some subjects Rh_1 give a weak reaction with anti Rh′ serum The antigen in the rbc is Rh_1^w (or C^wDe) A potent anti Rh^w (or anti C^w) serum is available to test this variant

Rbc which react weakly with anti-Rh′ serum but do not react with anti Rh^w serum constitute another variant

Variants of Rh_0 antigen—In some subjects Rh_0 is weak No specific antiserum (like anti Rh^w) is yet available to test them This antigen is 'Rh_0 variant' written $\mathfrak{R}h_0$ (Germanic R) For reactions and erythroblastosis fœtalis it is as potent as the strong Rh_0 (A S Wiener, 1950, *Brit Med Journ*, *i*, 813)

Rh IN OTHER CELLS

At one time the hemogen was supposed to be confined to the rbc only Later it was shown to be present elsewhere also (Kathleen E Boorman and Bárbara E Dodd, 1943, *J Path Bact*, **55**, 329)

TECHNIQUE OF TESTING BLOOD WITH HUMAN TESTING SERA

The suspension of rbc—It is made in the same way as in the technique for grouping blood or for testing with animal serum

The slide technique—Each slide is marked into 3 compartments on the obverse On the reverse are given along the lower border the designations of the antisera anti Rh_0, anti Rh and anti Rh″, beginning from the left

[These antisera are now known as (*i*) anti Rh_0 or anti D, (*ii*) anti Rh′ or anti C and (*iii*) anti Rh″ or anti E According to the latest modification in the

Again, for brevity, the homologous Rh positive parent is D/D, the homologous Rh negative parent is d/d and the heterologous positive parent is D/d (R N C McCurdy, 1950, THE RHESUS DANGER Its Medical, Moral and Legal Aspects William Heinemann Medical Books Ltd, London) For homology and heterology see the chapter on genetics

Really CDe as established by the three antisera may be in genetic constitution CDe/CDe, CDe/Cde, CDe/cde or Cde/cDe It was for the purpose of establishing the genetic constitution that the CDE system was introduced

Other human antisera—They are anti c, anti d (has not convinced A S Wiener, 1950, *Brit Med Journ*, *i*, 813) and anti e They are not freely available yet specially the last two

In a subject CDe the presence of e is (*i*) concluded from the lack of reaction of his rbc with anti E serum and (*ii*) established positively by the reaction of his rbc with anti e serum

Further, the fact that the antigen represented by a capital letter is present does not mean that the one represented by the corresponding small letter is absent

The 3 additional antisera, anti c, anti d and anti e of Fisher are really the 3 anti Hr sera, anti Hr′, anti Hr_0 and anti Hr″ of Wiener

One serious objection to the CDE system, as pointed out by Wiener, is that in it are introduced many letters and symbols, including 6 Greek letters for 6 antisera, but none of them has any relation to the symbol Rh

The complexity of the Rh system is not necessarily of a higher order than that of OAB and MN systems Had the system remained in the hands of serologists or biologists it would have settled down like the other systems which have their subdivisions and their anomalous reactions Mathematicians into whose hands it has fallen intend imputing to it a mathematical

The tube technique—Three small test tubes (as used in testing with animal serum or WR) are charged, thus —

	1st tube	2nd tube	3rd tube
Anti Rh_0 serum	3 drops	*Nil*	*Nil*
Anti Rh′ serum	*Nil*	3 drops	*Nil*
Anti Rh″ serum	*Nil*	*Nil*	3 drops
RBC suspension	3 drops	3 drops	3 „

Standard drops are used The tubes are shaken and left in the incubator for 1 hour The results are read over the concave mirror of the microscope (as in testing with the animal serum) They are clear cut as a rule Besides, the deposit of unagglutinated rbc puts out a tongue on tilting the tube

As in the case of the slide technique, one of the tubes is likely to act as the control

When all the tubes show agglutination a separate *control of the rbc will be necessary* Preferably, then, 2 controls should be prepared one in saline (rbc suspension 3 drops + saline 3 drops) and one in subject's own serum (or *in the serum of a subject of the same group or a* subject AB rbc suspension 3 drops + serum 3 drops)

If granularity is suspected in the tongue put out by *a deposit, a 1 mm loopful of the deposit should be* examined under the microscope

Attention has been drawn only lately to the granularity caused *by small clumps In testing blood* for transfusion, in excluding Rh incompatibility and in testing for iso immunization, minor grades of agglutination must be excluded

As often as not rouleaux are responsible for the granularity seen in the tongue

nomenclature the designation should be anti Rh_0, anti rh′ and anti rh″. As stated before, the author retains the capital letter R for the description.]

One standard drop of each serum is put into its appropriate compartment. Then a standard drop of the rbc suspension is added. The drops are mixed with the rounded ends of glass rods. The slides are placed in the moist chamber and left in the incubator for 1 hour. Every 15 minutes or so the fluid is agitated by moving the moist chamber in the usual way. Results are read at the end of 1 hour. They are clear cut as a rule. A hand lens or even a microscope may be needed at times. The small quantity of blood in each compartment of a slide does not touch a dry lens. After examination the slide is returned to the moist chamber. Even a partial drying of the preparation is immaterial for a microscopical examination: the spread of the unagglutinated rbc, the clumps of the agglutinated rbc and the rouleaux (when present) are characteristic. The rouleaux produce a granularity which to the unaided eye may look like a weak agglutination. The clumps may be *large* (8–12 rbc) or *small* (4–6 rbc) and *loose* (same colour as the rest of the rbc) or *tight* (distinctly deeper in colour). Even small and loose clumps indicate a ± reaction. Such a reaction has been designated W (Weak), coming after ± (A. E. Mourant, 1948, MEDICAL RESEARCH COUNCIL MEMORANDUM NO. 19. The Rh Blood Group, London: His Majesty's Stationery Office).

Usually only 2 compartments will show agglutination. The remaining compartment then will act as a control of the rbc.

When all the compartments show agglutination a separate control of the rbc will be necessary. Preferably, then, 2 controls should be prepared: one in saline and one in subject's own serum (or in the serum of a subject of the same group or a subject AB).

Special care necessary even with three antisera — The direct compatibility tests between the recipient's serum and the donor's rbc should be performed with special care in (i) transfusing mothers, expectant mothers and cases of complications of pregnancy and (ii) all second or subsequent transfusions, *even when the Rh tests with three antisera are satisfactory* The interference caused by Hr will be detected by an unexpected agglutination In terms of CDE, occasionally a D positive person may have formed antibodies against C or E, *or even against c or e*

The test should be set up both on slides and in tubes Observations should last 1 hour The slides and deposit in tubes should be examined under the microscope

Further, the donor's rbc should be tested in saline as well as in donor's own serum (see **Conglutination**)

To complete the list of precautions, the test should also include, if possible, the **indirect anti-human-globulin test (Coomb's Test)** This very delicate test for Rh antibodies of all kinds and the blocking anti-Rh_0 in particular is performed thus (i) leave a suspension of 2–5 per cent of the donor's rbc in contact with an equal volume of the recipient's serum for ½ hour at 37°C , (ii) centrifuge and discard the serum, (iii) wash the deposit 3 times in saline, (iv) re suspend the deposit to yield a 5 per cent suspension and (v) test 1 drop of the suspension with 1 drop of an appropriate dilution of rabbit anti human globulin serum, on a tile, after mixing well A drop of the rbc suspension is also tested with saline as a control The appropriate dilution of the rabbit anti-human globulin serum is determined by its reaction with known rbc There should be no agglutination of the rbc in the test or in the control

The conglutination test takes the place of this test if the necessary rabbit serum is not available

Specially prepared human Rh testing sera—Rh − human volunteers (type rh) are injected intramuscularly with the Rh + blood from subjects of the same group and type (O A B and M N) These sera yield the required antibodies against Rh_0, Rh′ or Rh″ Ideally the subject for iso immunization should be ABrh

As Rh′ and Rh″ are poor antigens the immunization is done with Rh_1 and Rh respectively and then the anti sera modified by an admixture of anti Rh_0 blocking body—see **Conglutination** This fact is stated on the leaflet accompanying the sera

Use of one antiserum only 1 Anti Rh_1 serum—This serum has been used alone The type of the rbc agglutinated by it may be one of the following —

(i) Rh_0, clinically positive

(ii) Rh_1, clinically positive

(iii) Rh_1Rh, clinically positive

(iv) Rh (= rh′), clinically negative

(v) Rh Rh″ (= rh′rh″), clinically negative

A positive report on (iv) and (v) would be incorrect

Further, an Rh + mother of one type may be iso immunized against another type It would not be safe to transfuse her boldly, simply because she is not Rh − The same remarks apply to a recipient of second or subsequent transfusion

2 Anti-Rh_0 serum—This serum is being used alone The type of the rbc not agglutinated by it may be one of the following —

(i) rh, serologically negative cde of the CDE system

(ii) Rh′ (= rh′), clinically negative

(iii) Rh′ (= rh), clinically negative

(iv) Rh′Rh″ (= rh rh′) clinically negative

Only (i) is the safe Rh − donor

Conglutination—Recently the importance of a blocking antibody has been revealed It is an agglutinoid which does not bring about hæmagglutination of rbc suspended in saline, but so sensitizes them that they do not agglutinate on the addition of a standard human anti Rh serum later Instead of being an ineffective hæmagglutinin it is a very potent hæmolytic agent in the presence of human serum

Its presence is detected thus The rbc used in the test are suspended in the serum of the subject himself or the serum of a subject AB On the addition of a serum containing the blocking antibody hæmagglutination develops in the usual way on slides and in tubes This is conglutination

The easiest way to suspend a subject's rbc in his own serum is as follows After removing the clear serum from the clot (in the tube intended for preparing serum) incise the clot and shake the tube Serum turbid with rbc will ooze out Separate the turbid serum in a small tube (Durham tube) Match the turbid serum with a standard 2 per cent rbc suspension Add more of clear serum to reduce turbidity, if necessary The turbid serum now is a 2 per cent suspension of the subject's rbc in his own serum No clumps of rbc should be seen

For transfusions in all maternity cases and for second or subsequent transfusions in all cases, the donor's rbc suspended in his own serum must be tested with the recipient's serum, in cross matching, *i e* a conglutination test must be done

This method is preferable to the use of whole blood in cross matching The whole blood under certain conditions coagulates and produces the appearance of hæmagglutination

Intra group and anomalous reactions, connected and unconnected with pregnancy, are known

Use of human anti-Rh serum—The serum unless derived from a subject AB is used for testing subjects

COLLECTION OF A HUMAN TESTING SERUM

An iso-immunized subject—The mother who has given birth to a baby suffering from erythroblastosis fœtalis is usually an Rh — subject who has become iso immunized by her Rh + fœtus (the fœtal blood escaping into the maternal circulation and immunizing the mother as the injected monkey blood immunizes a rabbit) Her serum will agglutinate rbc of most subjects, which otherwise should not be agglutinated *The serum should not be heated*

Tests for iso-immunization and suitability of a serum 1 When the mother belongs to group A— Test serum (on slides and in tubes) with 3 lots of rbc A and 3 lots of rbc O If there is hæmagglutination exclude the intra group reaction of the sub groups of A and then suspect iso immunization Continue tests until several negative reactions (no hæmagglutination) are obtained The suspicion is now confirmed Continue further tests until the expected Rh — rate is obtained The serum is now proved to be suitable for testing for Rh hæmogen

2 When the mother belongs to group B— Test serum with 3 lots of rbc B and 3 lots of rbc O If there is hæmagglutination suspect iso immunization and proceed as above

3 When the mother belongs to group AB— Test serum with 3 lots of rbc of any group If there is hæmagglutination suspect iso immunization and proceed as above

4 When the mother belongs to group O— Test serum with 3 lots of rbc O If there is hæmagglutination suspect iso immunization and proceed as above

5 Test with father's rbc—The father s rbc are suitable for the test in 2 cases out of 3, as the distribution of O A B in India is almost even When applicable this test is done first although it is given last

Conglutination—Recently the importance of a blocking antibody has been revealed It is an agglutinoid which does not bring about hæmagglutination of rbc suspended in saline, but so sensitizes them that they do not agglutinate on the addition of a standard human anti Rh serum later Instead of being an ineffective hæmagglutinin it is a very potent hæmolytic agent in the presence of human serum

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This method is preferable to the use of whole blood *in cross* matching The whole blood under certain conditions coagulates and produces the appearance of hæmagglutination

Intra group and anomalous reactions, connected and unconnected with pregnancy, are known

Use of human anti Rh serum—The serum unless derived *from a* subject AB is used for testing subjects

of the same group If its isomins can be neutralized (by substances A and B, obtained from saliva of secretors) it can be used for testing subjects of all groups It can be stored liquid, frozen or freeze dried

The serum is used undiluted, as in grouping blood The results obtained are clear cut ++, + and — (no ±) Both slides and test tubes are used

Sera containing blocking antibodies are also used for typing rbc (*see RAPID TESTS FOR Rh*)

TESTS FOR ISO IMMUNIZATION IN PREGNANCY

Tests as detailed for collecting human testing sera are undertaken in pregnancy also The facts that an expectant mother is Rh — and her husband Rh + do not mean that iso immunization exists

When an antibody is detected a monthly or fortnightly record is kept of its titre A rising titre may indicate induction of premature labour or cæsarean section

Rh antibodies when formed as a result of pregnancy are seldom detected before the 5th month Therefore, if detected earlier they indicate that the woman was sensitized before she became pregnant (P L Mollison 1948, MEDICAL RESEARCH COUNCIL MEMORANDUM NO 19, *loc cit*)

TESTS FOR ISO IMMUNIZATION AFTER REACTION OF TRANSFUSION

The reaction may reduce the titre of the antibody concerned in the hæmolytic reaction for a few days The tests must be repeated after a week or so

ADDITIONAL NOTES ON Rh ISO IMMUNIZATION

Every Rh — mother of an Rh — fœtus is not iso-immunized.—The escape of the fœtal blood into the maternal circulation may not take place The stimulus

even after an escape of blood, may not be strong enough. Usually an Rh − mother can have two healthy Rh + babies. Later, the repeated stimulus succeeds in producing the antibody.

An Rh − wife of an Rh + husband does not always carry an Rh + fœtus.—The reason is genetic. If the husband is heterozygous his gametocytes contain both an Rh + Rh, and an Rh −, rh, character. Some gametes will carry Rh and others rh. The gametes of the wife will of course carry only rh. The zygote resulting from gametes carrying rh will develop into an Rh − fœtus, in spite of the fact that the husband is Rh +.

A homozygous Rh + husband on the other hand produces gametes containing only Rh character (*see* chapter on genetics).

Biological test.—The test is performed as follows:—

(1) Take 10 cc. of the recipient's blood and divide into two lots in (*a*) a dry test tube and (*b*) a test tube containing 1 cc. of 3.8 per cent sodium citrate. Allow (*a*) to clot and centrifuge (*b*).

(2) Give intravenously 50 cc. of the proposed donor's citrated blood as a sample.

(3) After 1 hour take 10 cc. of the recipient's blood again and divide as before into two lots in (*a*) a dry test tube and (*b*) a test-tube containing 1 cc. of 3.8 per cent sodium citrate. As before allow (*a*) to clot and centrifuge (*b*).

(4) Compare the colours of the supernatant fluids of the wet tubes and the colours of the serum in the dry tubes.

Absence of colour denotes absence of hæmolysis. The wet tubes guard against a traumatic hæmolysis of the type which might occur in the dry tubes.

The biological test even if positive (the second sample of the serum showing a tinge of hæmolysis) will not do the recipient any appreciable harm It may, however, damage further a fœtus suffering from erythro blastosis fœtalis, by raising the titre in the mother's serum An unmarried Rh — female may be sensitized by the test in advance of an incompatible pregnancy

Iso immunization by human serum —Injection of serum or plasma into Rh— women may also give rise to Rh iso immunization and deprive even primiparas of the opportunity of having a normal baby A case has been described in which a young primigravida was presumed to have become iso immunized to Rh by the injection of pooled human serum, given as a prophylactic measure against poliomyelitis in childhood Her first baby developed fatal erythroblastosis (J T Wallace, A S Wiener and M H Dozle, 1948, *American Journal of Obstetrics and Gynaecology*, **56**, 1163)

Anamnestic reaction —An Rh negative woman previously sensitized to Rh antigens, by pregnancy or transfusion, may show anti Rh bodies in her serum, while carrying a normal and Rh negative fœtus The titre may even rise Cases have been recorded (Page, Hunt and Lucia, quoted by E L De Gowen and J B Alsever, 1949, BLOOD TRANSFUSION, W B Saunders Co, Philadelphia and London) The reaction is merely a revived memory of a previous reaction (ana = back + memnesko = call to mind) Other examples are known in immunology

Two Rh antibodies with an essential difference between them —The agglutinating antibody which agglutinates the appropriate rbc in saline is an agglutinin It has a large molecule and cannot pass the placental barrier The blocking antibody is an agglutinoid (or glutinin) which coats the appropriate rbc and prevents, later, the action of the agglutinin on them It has a small

molecule which allows it to pass the placental barrier into the fœtal circulation According to Wiener only this antibody is responsible for the disease in the fœtus in the presence of mature human plasma (This accounts for the development of the disease after birth or at full term) The titre of this antibody has been found to be directly correlated with the disease (A S Wiener Anniversary vol, *loc cit*)

O-A-B incompatibility plus Rh incompatibility in pregnancy—Rh incompatibility produces its effects less often when O A B incompatibility also exists (O A B compatibility exists when the husband can act as a blood donor to the wife)

Three explanations have been given (1) A competition occurs between the antigens In the competition the Rh antigen fails On this principle is based an attempt at treatment of erythroblastosis (*vide infra*) (2) The rbc incompatible in O A B when carried to the circulation is dealt with by the isonin and rendered inert before its Rh antigen can form an antibody (3) Elimination of the fœtus incompatible in O A B occurs at an early stage Obviously the Rh antibody will not be formed Combination father O and mother A has produced more children than the combination father A and mother O

DETERMINATION OF Rh HOMOZYGOUS/ HETEROZYGOUS STATE

Occasionally an Rh + mother gives birth to a baby suffering from erythroblastosis fœtalis She provides an anti Hr serum or St serum It contains an antibody against the Hr′ hæmogen which is associated with character rh

The serum agglutinates the rbc of all Rh — subjects and all heterozygous Rh + subjects It is a rare serum, though not so rare as other anti Hr sera

DETERMINATION OF OTHER CAUSES OF ERYTHROBLASTOSIS FŒTALIS

Occasionally incompatibility of blood groups gives rise to erythroblastosis fœtalis The titre of the isonin in the mother's serum for the isogen in the rbc of the fœtus is found to be very high (of the order of a thousand) After delivery the titre falls

Other incompatibilities, M, N and P, etc, have not attracted attention so far They may also operate

RAPID TESTS FOR Rh

Rapid Slide Tests with Blocking Antibody

For Rh antibodies in the serum—Known positive and negative bloods should be available Oxalated whole blood, for preference, or a heavy suspension of rbc in serum, alternatively, is used 2 3 drops of each blood and a drop of the serum under test are mixed on a slide which is warmed and observed for 3 minutes Hæmagglutination occurring in masses leaves no doubt of a positive reaction Weak and negative reactions are difficult to differentiate

The warming of the slide is done on a small box enclosing an electric bulb and fitted with a piece of ground glass in a window on top The slide rests on the ground glass (A E Mourant, 1948, MEDICAL RESEARCH COUNCIL MEMORANDUM NO 19, *loc cit*)

There should be no occasion to employ this method for clinical purposes

For rbc—With a drop of high titre known serum on the slide are mixed 2 3 drops of whole blood (oxalated or heparinized) or of a heavy suspension of rbc in subject's own serum The slide is warmed and watched for 3 minutes If masses of hæmagglutination are not formed within this time the reaction is negative

All 3 antisera may be used in this way

This method is likely to be used for anthropological surveys

Capillary Test with Blocking Antibody

Steps—(1) A capillary tube is dipped in the anti Rh serum to suck about ½ inch of serum and left (on the top of a pair of Petri dishes in which capillary tubes are sterilized and kept)

(2) The subject's thumb at the root of the nail (cleaned with alcohol which is allowed to evaporate) is punctured and a small drop of blood pressed out

(3) Near the drop of blood is placed a drop of 3 8 per cent sodium citrate solution

(4) The two drops are mixed with the stem of the capillary tube

(5) The mixture is allowed to enter the capillary tube and form a continuous column with the antisera (no air bubble)

(6) The capillary tube is inverted and set in plasticine at an angle of 45 degrees It rests either on a specially constructed warming box with a piece of opal glass inclined at an angle of 45 degrees and fixed in a window on top, or is incubated, and is examined in 5 minutes The difference between agglutinated and unagglutinated the in the column of blood should be obvious This is a positive result For a negative result the capillary tube should be incubated for 30 minutes (after Edith L Potter, 1948, Rh, The Year Book Publishers Inc, Chicago)

This method is also likely to be used for anthropological surveys

ERYTHROBLASTOSIS FOETALIS

Manifestation of the disease—The damage to the foetal rbc and organs manifests itself in 4 ways (1) Late progressive anaemia The infant appears to be normal at birth but a week or two later shows increasing pallor

A slight jaundice may or may not be seen The spleen is rarely enlarged Nucleated rbc in the blood stream do not attract attention in a stained film Blood transfusions are necessary Prognosis is very good Probably many cases in this category are missed (2) Early progressive anæmia and jaundice The infant born normal shows jaundice during the first day of life Nucleated rbc are seen The spleen may or may not be enlarged *Jaundice deepens for a few days* Transfusions are usually required Prognosis with treatment is good The critical period is 3–5 days (3) Severe anæmia and jaundice Jaundice is apparent within a few hours of birth Ecchymotic spots may be present The nucleated rbc attract attention The spleen and liver are enlarged Lethargy attracts attention Face and extremities may be slightly œdematous The placenta may be hypertrophic Prompt treatment will save many children Jaundice may persist for weeks (4) Generalized œdema Pleural and abdominal cavities may contain much fluid The nucleated rbc show many immature cells (erythroblasts) The spleen and liver are enlarged The placenta is œdematous Usually there is no jaundice Such infants die during delivery or shortly afterwards

Other causes of jaundice are (1) Congenital obliteration of the bile duct The jaundice usually occurs in the 2nd week although stools are acholic from birth There is no anæmia or erythroblastæmia (2) Congenital syphilis Erythroblastæmia may be present Skin lesions are likely to be present (3) Severe sepsis The jaundice occurs towards the end of the week Fever, etc, are present (4) *Familial acholuric jaundice* Some cases can begin early enough to be confused with the hæmolytic disease caused by the Rh incompatibility Osmotic fragility of the rbc is present

Hemorrhagic disease of the newborn because of the similarity between the words Hæmolytic and

Hæmorrhagic can cause serious confusion The hæmolytic disease may be complicated occasionally by hæmorrhage The essential defect in the hæmorrhagic disease is the low prothrombin level Whole blood injections or vitamin K are used Before whole blood is given the Rh type of the recipient must be determined

Pathological findings —A fœtus dying during early pregnancy may not show any characteristic changes When death occurs *in utero* later, or within a few weeks of delivery, the skin is usually macerated, the face œdematous and the tongue protruding The spleen and liver are enlarged Extramedullary hæmatopoiesis is present The heart is usually slightly hypertrophied In the infant dying after birth marked icterus of all tissues and fluids is seen Bile pigment is deposited in the basal nuclei, KERNICTERUS, and other parts of the brain, and in the renal pyramids There is focal necrosis in the white matter of the brain

All lesions can be traced to the damage to the liver from abnormal hæmopoiesis The abnormally functioning tissue of the liver degenerates and the rest (jaundice and œdema) follows (M N Richter, 1948, PATHOLOGY, edited by W A D Anderson, St Louis, The C V Mosby Company, U S A)

Nucleated rbc in blood film —They are looked upon as normal if under 10 per cent of the cells containing nuclei include wbc (The upper limit of nucleated rbc is 1,000 per c mm and of the wbc 10,000 per c mm)

Remote sequelæ —Infants who survive suffer permanent damage from (1) Effects of bile on the central nervous system Kernicterus and focal necrosis in the white matter of the brain leave behind defects of muscular tone, athetosis, diplegia and feeble mindedness (2) Effects of hæmolysis and thrombosis in the liver and spleen These organs are enlarged (3) Effects of anæmia on the general growth at a critical stage

Congenital malformations are commoner in such children than in normal children In this group are also included children whose mothers' blood have continued antibodies —anti Rh, or anti A and anti B stronger than normal— against the children's rbc The malformations include harelip, cleft palate, spina bifida, cervical rib super numerary digits, hydrocele, urethral stricture and hydrocephalus

Effects on the mother —Carrying of an erythroblastic fœtus appears to cause toxæmia in the mother if the disease in the fœtus is severe (Mollison, *loc cit*)

Treatment of the mother for the benefit of the baby —TAB vaccine has been introduced as a competing antigen In a case reported in India the expectant mother was given the vaccine (T 1,000 million, A 500 million and B 500 million per cc) subcutaneously and weekly from the 31st week of pregnancy A beginning was made with 0 25 cc and the dose was increased every week by 0 25 cc until 1 0 cc was reached The last dose was repeated 3 times In all 7 injections were given (0 25, 0 5, 0 75, 1 0, 1 0, 1 0 and 1 0) There was reason to believe that the baby was benefited although it died during a blood transfusion *via* the fontanelle (K S Ranganathan and Sher Singh, 1948, *Indian Med Gaz*, **83**, 117)

PREMATURE TERMINATION OF PREGNANCY IN Rh INCOMPATIBILITY

Induction of abortion —When an Rh − wife of a homozygous Rh + husband has had a still birth, all future labours are likely to be disappointing The question of terminating her fruitless pregnancies in the first three months may be considered

Induction of premature labour and transfusion — Birth before full term seems to improve the infant's chance of recovery from the effects of erythroblastosis

fœtalis Induction of labour at 37th-38th week is considered to be the most favourable compromise between the fear of the hæmolytic processes and the hazard of prematurity Even the 35th week may be considered when a severely affected baby has been born before

After birth the diagnosis of the disease in the baby must be established at once by examining the cord blood A film is made (for nucleated rbc) and some blood is taken in a dry test tube containing dry anti coagulants (for hæmoglobin level, bilirubin content, Rh type of the rbc and Rh antibodies in the plasma)

Arrangements must be made in advance for a transfusion Whether to transfuse or not depends on (1) Presence of jaundice at birth Colour of the cord and of the cord blood is important This is the most valuable sign (2) Degree of anæmia This also appears to be a good guide Hb value below 80 on the day of birth means a severe affection (3) Degree of erythroblastæmia A negative finding is unreliable A positive finding indicates severe affection (4) Degree of sensitization of the infant's rbc It is not a reliable finding (5) Presence of the antibody in the plasma It is not a reliable finding

Although as a rule each succeeding infant in a family tends to be somewhat more severely affected than the last exceptions also occur On the whole the majority consists of moderate and severe cases Such cases are likely to benefit from immediate treatment A minority consists of mild cases that do not need immediate attention and may not need transfusion at all

Œdematous babies are not likely to survive with treatment

See *BLOOD TRANSFUSION* in the chapter on clinical application of blood characters for details of procedures for transfusing babies

Rh INCOMPATIBILITY IN MARRIAGE

Advice before marriage.—Probably such advice will not be required in love marriages. Further, the risk of sensitization is low: only 1 woman in 20 will become sensitized. Furthermore, the woman will be able to bear 1 or 2 babies before she is sensitized.

In arranged marriages the Rh +/— state may be given precedence over the horoscope, etc.

Artificial insemination from a donor (AID).—When the children that could be borne have died and the family desires another child, this measure could be considered. The donor may be selected to resemble the husband in appearance and blood characters other than Rh. He should be known to the medical adviser only. He should not know the mother or the family he is donating his semen to. Of course he should not fall within the relationship forbidden by the communities concerned for marriage.

Two special difficulties arise: (1) Rh — population being small the selection of the donor is limited. (2) If many inseminations are done from the same donor provisions must be made to prevent marriages between half brothers and half sisters of whom he is the father.

For medicolegal complications of AID see chapter on forensic application of blood groups.

V

OTHER HÆMOGENS

1 P + AND P − TYPES

Besides O, A, B, C, M, N, S, Rh and Hr there are other antigens in the human rbc G, H and P have been described Of these only P has been studied in some detail, as it was found interfering with the study of M-N system

The presence of the hæmogen in the rbc is determined by the use of immune animal sera Occasionally human sera containing weak anti-P agglutinins have been found The agglutinins occur naturally or as a result of iso immunization They have not, so far, been held responsible for hæmolytic reactions of transfusion

P is not linked to O A B, M-N or Rh Hr system

Neither the antigen nor the antiserum is freely available yet

The possibility of P should be kept in mind while investigating an anomalous hæmagglutination

2 LEWIS + AND LEWIS − TYPES

The anti Lewis serum was obtained from a Mrs Lewis in England in 1946 The rbc of about 25 per cent English people were agglutinated by it

The antigen was designated Le and is now divisible into Le^a and Le^b

There is no linkage between this antigen and other hæmogens An interesting observation is that Le^a positive subjects have been found to be non secretors of isogens A and B (R Grubb and W T J Morgan, 1949, *Brit Jour Exper Path*, **30,** 198)

Rh INCOMPATIBILITY IN MARRIAGE

Advice before marriage—Probably such advice will not be required in love marriages Further, the risk of sensitization is low only 1 woman in 20 will become sensitized Furthermore, the woman will be able to bear 1 or 2 babies before she is sensitized

In arranged marriages the Rh +/− state may be given precedence over the horoscope, etc

Artificial insemination from a donor (AID) — When the children that could be borne have died and the family desires another child, this measure could be considered The donor may be selected to resemble the husband in appearance and blood characters other than Rh He should be known to the medical adviser only He should not know the mother or the family he is donating his semen to Of course he should not fall within the relationship forbidden by the communities concerned for marriage

Two special difficulties arise (1) Rh − population being small the selection of the donor is limited (2) If many inseminations are done from the same donor provisions must be made to prevent marriages between half brothers and half sisters of whom he is the father

For medicolegal complications of AID see chapter on forensic application of blood groups

VI

CLINICAL APPLICATION OF BLOOD CHARACTERS

BLOOD CHARACTERS AND DIATHESIS

No constitutional, neoplastic or infective disease is linked to the blood characters as such All diseases occur in all groups (O A-B) and types (M-N) at the same rate Rh — state carries special risks in receiving a transfusion of blood and in marriage only

Diathesis in a parent may be carried by the chromosome carrying the blood character and may be linked to the blood character in this way only Even this linkage has not been proved to be very significant

BLOOD CHARACTERS AND GRAFTS OF TISSUES, ETC

Skin grafts are known to take better if the blood group of the donor is compatible with that of the recipient Presumably the same consideration of compatibility would apply to all blood characters and all grafts

In inducing therapeutic malaria by injecting blood intravenously it has been found that incompatible blood prolongs the incubation period Incidentally, intramuscular injection of blood for this purpose is safer and equally effective

BLOOD CHARACTERS AND ERYTHROBLASTOSIS FŒTALIS

Rh incompatibility —The disease is now definitely associated with Rh incompatibility between the mother and the fœtus Usually the mother is Rh — and the fœtus Rh +, generally of the type containing the antigen Rh_0 Any type in the fœtus, however, can sensitize the mother if not present in her

3 DUFFY + AND DUFFY − TYPES

This is the latest addition to the hæmogens The antibody against it was formed after a blood transfusion (Marie Cutbush, P L Mollison and Dorothy M Parkin, 1950, *Nature,* **165,** 188, Elizabeth W Ikin, A E Mourant and Gertrude Plaut, 1950, *Brit Med Journ* i 584)

The antigen appears to be independent of O A B and Rh, and is present in about 66 per cent of the English population The following notation has been suggested

(1) *The system may be called Duffy (after the patient)*
(2) The antigen may be called Fy which is divisible into Fy[a] and Fy[b]

The antigen has caused a reaction of transfusion

Other hæmogens recognized in English Blood Banks are Kell (yielding Kell +/−) and Lutheran (yielding Lutheran +/−)

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The sensitization does not occur with the first or even the second pregnancy and does not occur in every case in which it can occur A previous blood transfusion, incompatible with respect to the Rh state, in a woman has the same significance as a pregnancy

Hr incompatibility—An Hr negative mother (Rh positive as a matter of course, *see* Rh BLOOD TYPES and Hr) can also be sensitized by an Hr positive fœtus

M-N incompatibility—The incompatibility has not attracted attention in practice

O A-B incompatibility—Erythroblastosis is known to be occasionally caused by the incompatibility in hetero specific pregnancies The titre of the mother's serum for the rbc containing the heterospecific isogen is then very high

As a practical therapeutic measure it is worth while to see the effect of weaning on a baby which is not doing well and is incompatible with its mother in O A B

In view of what has been said under sub groups of A, theoretically the fœtus can even belong to group O Further, both the mother and the fœtus can belong to group A (different sub groups)

Rh incompatibility present alone is more effective than when present in addition to O A B incompatibility A husband is not incompatible when he can act as a donor to his wife

BLOOD TRANSFUSION

Less than 10 years ago the transfer of the rbc was reckoned to be almost the sole purpose of the blood transfusion, although immunized donors and alkalinized donors were being used

The transfusion of plasma and serum (in their original state or reconstituted from a freeze dried powder) has been popularized only during the World

War II They have been found to be more beneficial and less risky than the whole blood, for most patients

The freeze dried serum was essentially a war product and probably will not be available on a large scale any more The natural pooled plasma, on the other hand, can be made available in every blood bank (*see* BLOOD TRANSFUSION SERVICE AND BLOOD BANKS IN INDIA)

Fresh whole blood can be (*i*) transfused direct from the donor to the recipient by a suitable pump which prevents regurgitation such as Soresi blood transfusion apparatus (made by J Sklar Mfg Co, New York) or (*ii*) collected first in a solution of sodium citrate and given intravenously, intraperitoneally or intramuscularly like normal saline It must be passed through several layers of gauze to remove minute clots which might have formed in spite of the citrate solution Recently fresh blood has been heparinized Either the donor is injected—1 mg per kilo of weight, in 5 per cent solution—or the substance is added to the drawn blood, instead of the citrate—20 mg per 500 cc of blood, 0 4 cc of a 5 per cent solution diluted with 10 cc saline (G E Beaumont and E C Dodds, 1947, RECENT ADVANCES IN MEDICINE, J & A Churchill, Ltd, London)

Stored whole blood as obtained from a blood bank can also be given like fresh blood Both transfers require (*i*) previous determination of the blood characters of the donor and the recipient and (*ii*) careful cross matching unless in an emergency a safe universal donor is being used The same observations of caution apply to the use of blood of other groups for a recipient AB

For a distinction between **stored** and **preserved** blood *see* APPENDIX I, *ADDITIONAL NOTES*

The **dose** is controlled by the finding that 15 cc of normal blood for every kg body weight increase the rbc count by 1 million per cubic mm For an average

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European adult of 70 kg (about 150 lb) the dose by this finding should be about 1,000 cc Actually the usual dose is 500 cc In India for most cases it will be smaller (300–400 cc) Children can receive relatively more blood than adults Small volumes given frequently may suit some recipients more than a single large volume

The **rate of flow** for fresh blood, given direct from the donor to the recipient, with a special contrivance, is 50–100 cc per minute At a slower rate the blood coagulates For citrated blood a rate of 10 cc per minute will be found satisfactory in most cases It may be higher in cases of sudden hæmorrhage when but for a transfusion the life would be lost The **continuous drip** method (30–60 drops per minute) is hardly suitable for Indian dust, humidity and heat to which the blood would be exposed, in the plains, for the major part of the year It has, however, been advocated The **state of the right side of the heart** is important Signs of failure contra indicate a blood transfusion The continuous drip, not exceeding 30 drops per minute, may, however, be attempted

Attention has recently been drawn to a mechanical danger in this form of transfusion If the rubber tubing connecting the patient's vein becomes detached from the bottle of blood and falls to the floor the patient will be exsanguinated The accident may not be observed or may be observed only by the patient who may be too ill to call out

Limit of incompatible blood given in error, intra venously, has been placed at 100 cc Fatal accidents, however, have been reported from 5 cc given intravenously for therapeutic malaria

In intramuscular transfusion given to infants the group of the donor may be disregarded if the quantity transfused does not affect the volume of the infant's blood appreciably

Blood characters of the donor—When the plasma (or serum) is available there is no excuse for a hurried whole blood transfusion The best donor is the one who has the same group, type and Rh state as the recipient The type (M N) may be ignored The Rh state is important

Even an animal antiserum gives a good differentiation between the Rh + and Rh – groups without disclosing the actual types An Rh – recipient should never be transfused with an Rh + whole blood Further, for the purpose of transfusion, a ± reaction in the recipient should be taken as – and in the donor as +

Apart from the Rh state incompatibility within the same group may result from (1) Sub groups of A (2) Abnormal iso hæmagglutinins which may arise from (i) the action of previous transfusions on cold iso hæmagglutinins, (ii) morbid state itself, and (iii) pregnancy

Serum incompatibility due to other causes—Such causes are possibly allergic A scratch test on the recipient with the donor's serum, and a complement fixation test between the serum of the recipient and that of the donor are advisable, when a transfusion reaction, howsoever slight, is deemed to be particularly undesirable

Reactions of transfusion 1 Failure of the heart—This results from overloading of the circulation by too much blood or too rapid injection and is probably the most dangerous consequence of blood transfusion, particularly in patients with damaged hearts The mortality from direct transfusion, donor to patient (from donor's vein to the recipient's vein), which must be finished quickly, is higher than that from the transfusion of citrated blood which can be given more slowly

The symptoms occur during the transfusion or within an hour afterwards, and consist of extreme dyspnœa,

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The symptoms occur during the transfusion or within an hour afterwards, and consist of extreme dyspnœa,

orthopnœa and cyanosis Bloody froth may be coughed up Jugular veins are distended Lungs are full of rales Cardiac rhythm may be normal or abnormal The patient may die within a few minutes of the appearance of the symptoms If he survives without treatment, peripheral œdema may develop The only condition causing difficulty in diagnosis is asthma

This emergency cannot be treated by drugs Tourniquets should be applied to the proximal portions of the four extremities to occlude veins only They will divert about 15 per cent of the circulating blood from the heart This measure is a first aid treatment as well as a diagnostic test In the meantime preparations should be made to remove the overload by bleeding

The patient is then watched for several hours for œdema of the lungs and cardiac arrhythmia The former should not occur if the bleeding has been sufficient and the latter will need drugs The volume of blood removed should equal the one transfused

The overload is not due to the transfusion of whole blood only It is caused by plasma, substitutes for plasma and saline also The volume of blood removed for saline may be less than the one given

Tourniquets should be handy when the heart is suspected Four rubber tubes long enough to go round the upper arms and thighs will suffice No tourniquet should remain in position for longer than 20 minutes at a time All should be loosened by turn if a longer period is necessary for observation or operation

2 Damage to the rbc—Immunological reactions between the rbc and serum cause agglutination and hæmolysis in cases of incompatibility The first may kill by embolism or thrombosis and the second by the toxic action of the freed hæmoglobin on the kidneys particularly The various incompatibilities of blood

groups, blood types (possibly) and Rh state are responsible for the reaction

There are **three stages** **(1) During the injection** The patient complains of pain in the head, sense of oppression in the heart, tingling and pain in the lumbar region Inspection shows suffusion of the face, dilatation of veins in the neck, restlessness and dyspnœa There is cough and the expression shows anxiety The transfusion must be stopped and ½–1 cc of an adrenalin hydrochloride solution (1 in 1,000) administered if there are signs of collapse The dose may be repeated if necessary This stage cannot be recognized when the patient is under an anæsthetic

(2) Within an hour of the immediate reaction There is a chill followed by a rise of temperature The urine passed is dark red in colour with free hæmoglobin A general hæmorrhagic tendency may develop

(3) Apparent improvement and renal failure In cases that survive these reactions there follows a period of apparent improvement excepting suppression of urine This is the beginning of the renal failure and calls for appropriate medical or surgical treatments About half of the cases recover with an abrupt onset of diuresis

The medical treatment consists of inducing diuresis (intravenous injection of dextrose and saline solution alkalies by mouth—10 grams of sodium bicarbonate 4 times a day—and cupping over kidneys) The surgical treatment in desperate cases consists of decapsulating the kidneys

If sulpha drugs have been used it may not be easy to say what has caused the renal lesion The colour of the serum and a stained blood film showing phagocytosis of rbc will decide

The morbid process depending on the damage to the rbc may end favourably at any stage Not all cases of incompatibility suffer from a reaction

3 **Allergic shock** —Symptoms of varying severity supervene including urticaria Adrenalin is useful in combating them The donor should be free from all suspicion of allergy and idiosyncrasy and should donate blood while fasting Storage of blood also reduces risk of allergy

Urticaria and local swellings have been noticed in children after intramuscular transfusion of blood

A new danger Homologous serum jaundice — This recently recognized danger in England should make one mindful of unnecessary transfusion of whole blood, plasma or serum (The jaundice does not depend on the isogen and the isonin, and is much more than a mere jaundice isoserum hepatitis would be a better name) No previous treatment of the serum (which can infect in minute doses—0 01 cc) is effective The infective agent is probably a virus but is different from the virus of the ordinary infectious hepatitis (*see* APPENDIX II, HOMOLOGOUS SERUM JAUNDICE)

Blood transfusion for babies suffering from erythroblastosis fœtalis 1 When the disease is not suspected before birth —(1) Ideally an Exsanguination Transfusion from a compatible and Rh — donor should be given Blood volume should be calculated from the weight of the baby, about 50 per cent above the quantity transfused and an equal quantity removed at the same time The whole blood in adults is about 1/11th of the body weight In infants it is more, say 1/10th In this procedure most of the baby's blood and the Rh antibodies present in it are removed The new blood formed by hæmopoiesis will not be attacked The transfused rbc will function until new rbc are formed (2) As a second best measure, small quantities of compatible Rh + and Rh — blood in small lots (20 cc + 20 cc daily for Indian babies) for 3 days will suffice The former will neutralize the antibody and the latter will function until the new blood is formed **Mother's whole blood is not**

to be used because of the antibody it contains Rh- only can be used if compatible (3) As an emergency measure blood from any safe universal donor will suffice In the meantime arrangements are made for more appropriate donors (from *Indian Med Gaz*, **81**, 243 Adapted from W W Hallwright, 1946, *New Zealand Med J*, **45**, 2)

Wiener for practical purposes takes the blood volume of a newborn infant, weighing 7 lb, to be 250 cc He (i) exposes the internal saphenous vein at the ankle, (ii) ties in a blunt 20 gauge cannula with catgut, (iii) administers 500 units of heparin (in ½ cc of Upjohn's preparation) and (iv) commences transfusion of blood by the drip method Next he (v) exposes the radial artery at the wrist, (vi) introduces a short bevel 20 gauge needle and (vii) starts a flow of blood from the artery By the time this flow starts about 50 cc of blood will have entered through the vein The flow of transfusion is (viii) regulated to keep up this advantage of 50 cc of blood When 400 cc of blood are drawn (ix) the artery is ligated When 500 cc of blood have been given (x) the vein is ligated A second dose of heparin can be given if the flow from the artery slows and stops The whole operation took 90 minutes in the first case described (A S Wiener and I B Wexler, 1946, *Jour Lab & Clin Med*, **31**, 1016)

2 **When the disease is suspected before birth** — The plan given above is modified as follows (i) The serum from the mother is examined monthly, after the 5th month of pregnancy, for the rising titre of the Rh antibodies (agglutinin as well as agglutinoid) (ii) Cæsarean section is considered when the father is homozygous Rh positive (iii) The colour of the umbilical cord and of the plasma of the cord blood is examined immediately on birth and if found icteric (in an Rh + baby) a blood transfusion (of a suitable blood) is given without waiting

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pyrogen to cause febrile reactions The bacteria can be killed by exposure to steam at 121°C for 20 minutes or to dry heat at 180–200°C for 60 minutes These measures, however, do not destroy the pyrogens already formed For all practical purposes (i) the use of thoroughly cleaned and sterilized apparatus and (ii) immediate sterilization of quickly distilled water will provide pyrogen free water When water so obtained is used in washing mother still and flask, before sterilizing them for use, the absence of the pyrogen is doubly assured

Sometimes non bacterial substances like denatured blood proteins, etc, i e also called pyrogens is they can produce febrile reactions Traces of blood proteins can be removed easily by thorough washing and digestion with hydrochloric acid (*see* Preparation of Glassware, etc)

Exchange transfusion, in babies, via the umbilical vein—The apparatus consists of (i) a special plastic catheter and (ii) a system of one syringe, two 3 way stopcocks and one 19 gauge needle The assemblage runs in this order The syringe, 1st stopcock, 2nd stopcock, the needle and the catheter The syringe, the needle and the catheter are in a line, and the two arms of the stopcocks at right angles to the line one arm is connected with a bottle of blood and the other carries a rubber tubing leading to waste

The catheter mounted on the needle is introduced into the umbilical vein and guided into the inferior vena cava The syringe is then rinsed in heparin saline (1 cc of heparin—1,000 Toronto units—in 100 cc saline) attached to the system, filled with the baby's blood and emptied via the waste tube It is filled from the bottle next and emptied into the baby via the catheter The process is repeated The syringe is changed after a few transfers of blood 400–500 cc are exchanged in $\frac{3}{4}$–$1\frac{1}{2}$ hours The lower figure will suffice for Indian babies

for the appearance of the clinical symptoms of erythroblastosis fœtalis (from *Indian Med Gaz*, **82**, 76 Adapted from D F Cappell, 1946, *Brit Med Journ* ii, 641)

Additional Notes on Blood Transfusion

Transfusion of whole blood without anti coagulants—Whole blood does not coagulate in a container which it cannot wet Paraffined tubes have been used Containers made of athrombit have also been used (they do not appear to have become available freely) The best device is a pump (such as Soresi blood transfusion apparatus mentioned before) which takes blood from a donor in small quantities and injects it into a recipient and in which chances of regurgitation are eliminated (minimized)

There is really no merit in giving blood direct Sodium citrate contained in 500–1,000 cc is not likely to interfere with metabolism

Reactions from sodium citrate—The toxicity of this salt (when an untoward reaction does occur) is now traced to pyrogens which can contaminate the crystal during its formation The manufacturers should be asked to give an assurance that the possibility of pyrogen formation as eliminated

Theoretically, the salt can bind calcium in the recipient's plasma and thus cause tetany and hæmorrhage Both these occurrences can be overcome easily by intravenous calcium gluconate (up to 10 cc of a 10 per cent solution) given separately

The failure of blood to clot in a shocked patient is due to elaboration of heparin by the liver, not to the sodium citrate in the transfusion (if one has been given)

Pyrogens—These substances, probably carbo hydrates, are products of certain non pathogenic bacteria found in river water and dust They can grow in distilled water and produce in 15–18 hours sufficient

Simple transfusion, in babies, via scalp veins — The temporal areas are shaved and cleaned, and a fine 24 gauge needle with a very short bevel is introduced into a superficial vein With the aid of a 3 way stopcock blood is drawn from a bottle into a syringe and injected into the veins slowly If no hæmatoma forms all is well Otherwise another vein must be selected The operator who introduces the needle remains in charge of the needle only The usual test of drawing blood into the syringe from the vein is omitted A little less than a cc can be injected in a minute The total quantity should not exceed 10 cc per pound of body weight (Mollison, *loc cit*)

Several small transfusions, instead of one big transfusion, are also useful

Transfusion via bone, muscle, peritoneum, etc — Of bones for infants the tibia appears to be the best The site for the needle is anteromedial and 2 3 cm above the condyle Local anæsthetic is used Intramuscular transfusion is not now in favour Intraperitoneal transfusion was always a risky and useless procedure

For older children sternum may be used, manubrium or gladiolus

Intracardiac transfusion is a hopeless undertaking

Veins for bleeding or transfusion —In addition to the veins in the cubital space, veins on the dorsum of hands and feet, veins at the ankles, the corpus cavernosum of the male and the external jugular veins are quite convenient The superior longitudinal sinus of the infant through enticing is not safe

Rh + mothers' erythroblastic babies —They must not be forgotten Appropriate blood should be given

For collection of blood, transfusion of plasma and plasma substitutes, etc, *see* Appendix I, THE BLOOD

(The catheter is made of 'Polyethylene' It is kept immersed in 1 per cent 'Cetavlon' and rinsed in sterile saline before use A plastic sleeving of the same material for insulating wires is available in England from The Telegraph Construction and Maintenance Co, Ltd, Telcon Works, Greenwich, London, S E 10, as '1 mm bore × 0 25 ml Wall Telcothene Sleeving' and can be used instead of the catheter)

The introduction of the catheter needs a little practice The umbilical vein can be used up to about 24 hours after birth After cleansing, the cord is cut across about ½ inch from the abdominal wall (P L Mollison, 1948, MEDICAL RESEARCH COUNCIL MEMORANDUM NO 19, THE Rh BLOOD GROUPS AND THEIR CLINICAL EFFECTS)

For the purpose of transfusion the infant recipient must be immobilized by means of appropriate swaddling and adhesive plaster

For exchange transfusion female donors appear to be superior to male donors (F H Allen, Jr, L K Diamond and J B Watrous, Jr, *The New England Journal of Medicine* Abstract in Medical Newsletter, Wa 260, May 1950, prepared by the American Medical Association)

Simple transfusion, in babies, via the umbilical vein—When facilities for exchange transfusion are not available, a simple transfusion is given by the umbilical vein The cord is cut as before and an ordinary cannula is tied into the vein

When the birth of the affected infant is expected and facilities for an exchange transfusion are not available, the umbilical cord should be clamped immediately the infant is born, with a view to stopping about 100 cc of Rh positive blood (with the Rh antibody) from entering the infant from the placenta The quantity of the transfused blood is also 100 cc (Mollison, *loc cit*) The figure will be lower for Indian babies

Need for keeping a detailed record of the recipient's rbc and serum before transfusion—These reactions should be noted in details All rbc do not show the same degree of agglutination Apart from the 4 grades [++, +(+), + and ±], some rbc do not appear to be affected much Their number may be nil, negligible or considerable

Rbc A differ markedly both in the speed and the quality of the agglutination From 'slow' A one suspects A

The serum is more variable than the rbc Its potency may be good (like that of the standard serum), fair (producing agglutination of a good quality after prolonged contact) or poor (producing poor agglutination even after prolonged contact)

M-N system is being ignored at present

The Rh group and type of the rbc must be recorded and the quality of the agglutination described The kind of antisera used (animal or human) and their number and designation should be stated, namely, (*i*) anti-Rh_0, (*ii*) anti Rh_1, or (*iii*) anti Rh_0 anti Rh′ and anti Rh″, and anti Hr—if used

(Any evidence of allergy in the subject or family should also be recorded Allergy can kill exactly like an incompatible transfusion and should be guarded against)

TRANSFUSION SERVICE AND BLOOD BANKS IN INDIA

Difficulties in Determining Blood Characters after Transfusion

When all has gone well 1 With a donor of the same group —No change in the antigen of the rbc and the antibodies of the plasma occurs only when both these constituents of the blood are identical in the donor and the recipient Otherwise (1) Alteration in the agglutinability of the rbc occurs and abnormal isonins appear in the plasma These changes probably result from ignoring the sub groups of A (2) Activation of cold agglutinins takes place This item has been considered previously (3) Anti M and anti N agglutinins may be formed Normally they do not occur in the human blood (4) Antibodies against Rh and Hr may be formed They may remain in the blood without causing any harm but will interfere with cross matching blood with a donor previously compatible (5) Rh + rbc may survive in an Rh — recipient and interfere with Rh grouping and typing

2 **With a donor of group O** —The surviving rbc O may give a false impression of the group of the subject

(After plasma substitutes—gelatine gum, etc —the agglutinability of the rbc is defective If the substitutes are being used as a subsidiary measure only and a blood transfusion is contemplated, specimens of the recipient's blood should be taken and preserved for cross matching, prior to the use of the substitutes)

When all has not gone well —The antigen content of the rbc and the antibody content of the plasma will disclose a mixture not found normally Rbc A and rbc B may be found together with isonins a and b Rh anti bodies may be found Immediately after a reaction, they may occur in a very low titre

VII
GENETICS OF BLOOD GROUPS, BLOOD TYPES, RHESUS GROUP, ETC

GAMETOCYTES, GAMETES AND ZYGOTE, ETC

The body cells, including the essential cells produced by the reproductive organs in the first instance, the

DIVISION OF CELLS

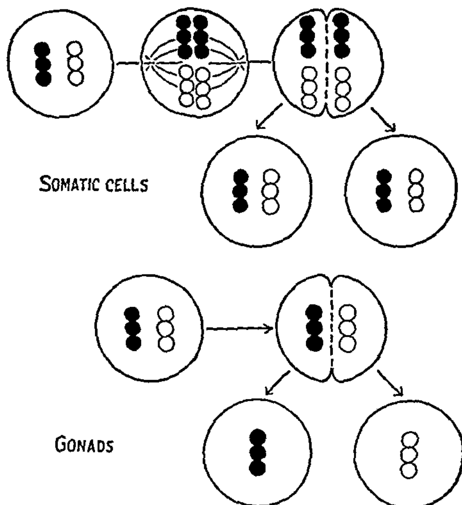


Figure 5 The somatic cells divide in such a way that the daughter cells have the same number of chromosomes as the parent cell

The gonadic cells divide like the somatic cells in the early division Later in the final stage shown in the diagram they divide in such a way that the daughter cells have only half the number of chromosomes characteristic of the somatic cells of the species

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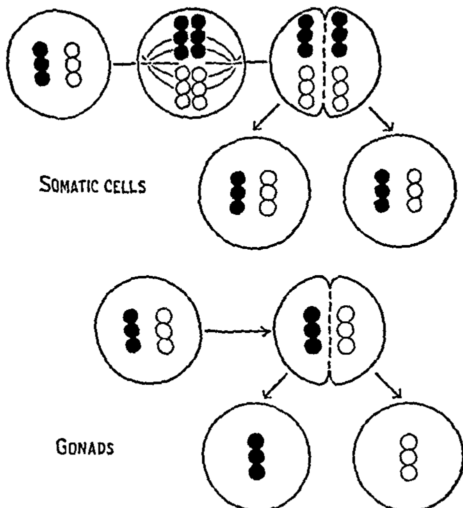


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GAMETOCYTES, have in their nuclei a definite and fixed number of pairs of chromosomes When a gametocyte forms two GAMETES, the latter inherit only one member of a pair of chromosomes each This is the REDUCTION of chromosomes, which always occurs when gametes are formed In the reduction the original 2n or DIPLOID number is reduced to n or HAPLOID number

In the foregoing figures the chromosomes are shown as consisting of one pair only, as in *Ascaris megalocephala*, variety *univalens* In the American fruit fly, *Drosophila melanogaster*, on which enormous work has been done, there are 4 pairs

In man there are 24 pairs Aid to memory

STRINGS OF HUMAN CHROMOSOMES

DOUBLE SINGLE

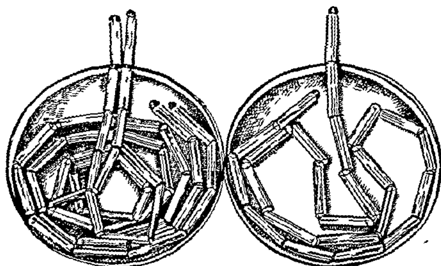


Figure 6 From glass models Note 24 chromosomes in each string

When fertilization occurs, two gametes (male and female) fuse to form a ZYGOTE In the zygote the reduced number of the chromosomes is restored and from the zygote grows a new body characteristic of the species

In the cells of the new body one member of a pair of chromosomes is derived from one parent and the other from the other parent

A chromosome is built up of a number of segments, the CHROMOMERES Each chromomere probably carries the physical basis of an attribute or a CHARACTER found in the species, the GENE A gene from the father on one chromosome lies at a LOCUS opposite the corresponding gene from the mother on the other chromosome, on the corresponding, HOMOLOGOUS, Locus The genes in apposition exist as opposite numbers and do not fuse They separate when the reduction of chromosomes occurs once again The separation, thus, separates, SEGREGATES, the characters

Chromosomes lie in apposition like the two members of a pair of socks in a box

When the characters are *contrasting* in nature (*e g* *brown* eyes and *blue* eyes) they are called ALLELOMORPHS

Crossing over of genes—Before separation the chromosomes are intertwined In the undoing of the intertwining they may break into one or more pieces which may change places as shown in the diagram

Thus certain genes situated on distant loci on one chromosome may CROSS OVER to a corresponding locus on another chromosome Another crossing over, DOUBLE CROSSING, may occur at another stage and restore the original association between the genes The composite Rh gene, in spite of its compositeness, occupies a single locus on the chromosomes and is not believed to be affected by crossings The genes which cannot separate are LINKED or held in LINKAGE

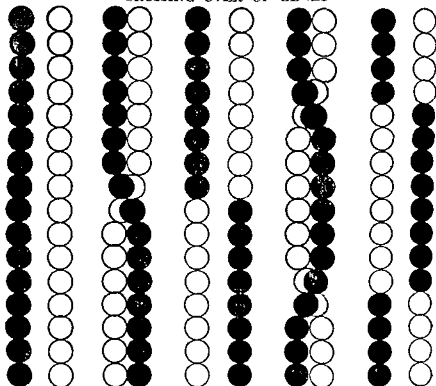


Figure 7 Extreme left chromosomes lying side by side second from left intertwining third from left lower halves exchanged fourth from left intertwining fifth from left three parts exchanged

From Textbook of Public Health by W M Frazer and C O Stallybrass 1948 E & S Livingstone Ltd Edinburgh (Original in The Mechanism of Creative Evolution G G Hurst Cambridge University Press) with permission

Haploids—A gamete instead of becoming a zygote by union with another gamete may build a body parthenogenetically Males of bees and wasps are derived from such gametes In frogs eggs can be 'fertilized' chemically or mechanically The individuals so developed are HAPLOIDS and the occurrence is HAPLOIDY

In lower plants the gametophytes (as opposed to the sporophytes in the alternation of generation) are regularly haploids Even in higher plants haploids have been found in several species of *Datura* and *Nicotiana* and in wheat and tomato The haploids generally resemble

the ordinary plants of their species, the DIPLOIDS, but are smaller and weaker in reproduction

Haploids can be induced by cold, radiation and other external changes

Polyploids—In the vegetable kingdom commoner than haploids are POLYPLOIDS, with more than two homologous chromosomes TETRAPLOIDS result from the union of gametes in which the reduction division has not occurred TRIPLOIDS result from the union of tetraploids and diploids They have *three* homologous chromosomes instead of *two* HEXAPLOIDS and OCTAPLOIDS are less common

The sex chromosomes XY plan—One of the pairs of chromosomes carries the genes of sex, X or Y X is like other chromosomes but Y is smaller Aid to memory *see* Figure 8 on the next page

Females produce gametes carrying X only, while males produce gametes carrying X or Y All ova, therefore, carry X Half the spermatozoa carry X and the other half Y

After fertilization

$$X + Y = \text{male and } X + X = \text{female}$$

The female is a female because it has *two* X's The male is a male because it has *one* X, not because it has a Y

The sex is carried only on one pair of chromosomes, the SEX CHROMOSOMES The other chromosomes are AUTOSOMES

In this instance the male produces different kinds of gametes Animals of this type are MALE HETEROGAMETIC

Non-disjunction—Sometimes the corresponding chromosomes of a pair do not separate in the reduction division This is NON DISJUNCTION One gamete then is short of a chromosome and the other has a chromosome in excess

The female gametocyte (on the left) contains XX Normally one X should be cast off in the polar body and

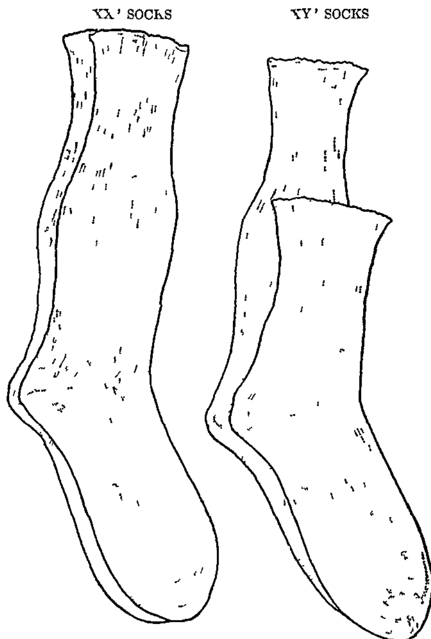


Figure 8 The pair under XX consists of socks of the same size (of foot) and the same leg length

The pair under XY consists of socks of the same size (of foot) but different leg length

one retained in the gamete (the ovum) Abnormally both X's may be cast off in the polar body or retained in the gamete because of non disjunction

NON DISJUNCTION

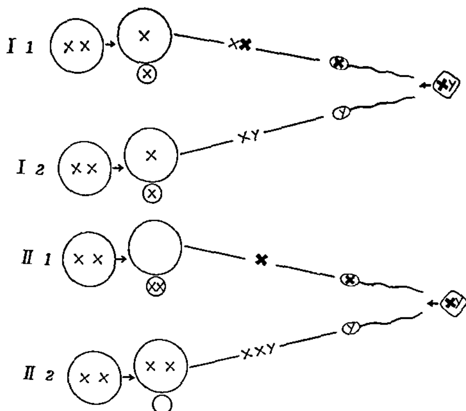


Figure 9 Formation of ova (on the left) and spermatozoa (on the right) Note the non-disjunction of XX in II 1 and II 2
The chromosomes from the ova and the spermatozoa meet in the centre of the diagram in the zygote (forming XX, XY X and XXY)
After E W Sinnot and L C Dunn Principles of Genetics' McGraw Hill Book Company Inc New York and London 1939 with permission

The male gametocyte (on the right) contains X and Y The gametes (spermatozoa) contain either X or Y

This explanation has been given in the irregular inheritance of red eyes by the male offspring of a white eyed female *Drosophila melanogaster* mated with a red eyed male

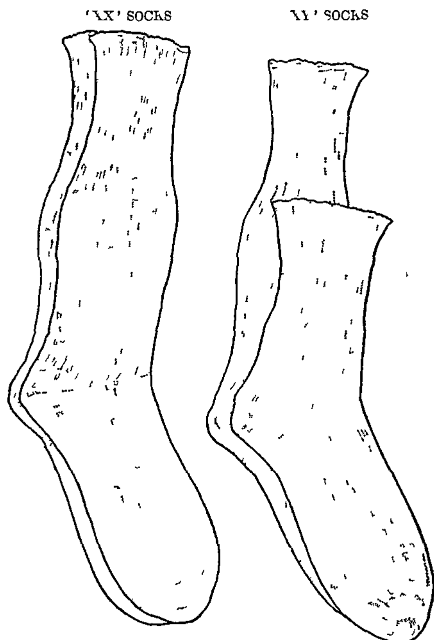


Figure 8 The pair under XX consists of socks of the same size (of foot) and the same leg length

The pair under XY consists of socks of the same size (of foot) but different leg length

Female HETEROGAMETY has been found chiefly in birds and moths

The two plans, male heterogamety and female heterogamety, do not exhaust the mechanism of differentiation of sex

MENDELISM

When the edible pea plant of a 'pure breed' (*vide infra*) of the tall variety is crossed with one of the dwarf variety, the offspring grown from the resulting peas are all tall The TALL character is DOMINANT and the d w a r f character r e c e s s i v e, thus

Female gametes \ Male gametes	d	d
T	Td	Td
T	Td	Td

T = Chromosome carrying tallness

d = Chromosome carrying dwarfness

In first generation of hybrids

T × d = Td = TALL

All are tall

When the plants of the first generation of hybrids are crossed, the offspring grown from the resulting peas are not all TALL As a matter of fact there are three TALL plants to one d w a r f plant

The reason is that in each pair of chromosomes in the gametocytes of the hybrid plant the genes for the TALLNESS and the d w a r f n e s s were lying side by side In the gamete there was included only one chromosome carrying either T(allness) or d(warfness) Two male gametes from one plant crossed with two female gametes from another plant produced the following combinations

	T	d
T	TT	Td
d	dT	dd

TT Td and dT = 3 TALL

dd = 1 dwarf

Normally, white eyed females mating with red eyed males produce only red eyed daughters and white eyed sons This is shown in the upper half of the figure I, 1 and figure I, 2 The colour of the eye is linked with X Thick X from the male carries the red colour of the eye It can be found only in the daughter (carrying two X's)

Abnormally, white eyed females mating with red-eyed males produce some red eyed sons and white eyed daughters This is shown in the lower half of the figure In figure II, 1 the ovum carries no X, both X's being discharged in the polar body, as a result of non *disjunction The zygote carries only what the* spermatozoa carried, namely, a thick X carrying red colour of the eye The result is a red eyed male (exceptional and sterile) In figure II, 2 the ovum retains both X's, again due to non disjunction They are thin and carry the white colour of the eye The result is a white eyed female (exceptional) Y of course does not count when two X's are present to produce a female

The colour of the eye is in this instance X linked the thick X carries the red and the thin X the white

Another plan of determination of sex ZW plan — The female produces different kinds of ova containing Z and W The male produces the same kind of spermatozoa, all carrying W

After fertilization

$$W + W = \text{male}$$
$$Z + W = \text{female}$$

Z and W might as well be called X and Y respectively, the point of difference being that animals of these types are FEMALE HETEROGAMETIC

In the domestic fowl the female lays eggs of two sorts half with X and half without X The male produces spermatozoa of the same kind all containing X

After fertilization

$$X + X = \text{male}$$
$$X + \text{nothing} = \text{female}$$

The symbol d is rather unorthodox In the usual description it is replaced by t (opposed to T) No useful purpose, however, is served by representing dwarfness by t

This law of inheritance was established by Rev Father Gregor Johann Mendel (1822 1884), Abbot of Brunn, and published in the Proceedings of the Natural History Society of Brunn Its significance as a biological phenomenon was dwarfed by Darwinism in 1858 The law was rediscovered by the botanists De Vries, Correns and Tschermak in 1900

TELEGONY

The term telegony is applied to a rare, doubtful, but, if true, remarkable mode of inheritance of characters It is the influence of a previous sire on offspring borne by the same female, later, to a different sire (J A Thomson, *HEREDITY*, 1907, John Murray, London, same author BIOLOGY FOR EVERY MAN, 1934, J M Dent and Sons, Ltd , London)

The race horse Blair-Athol had a very characteristic blaze (white bald face) The mares covered by him bore foals with the same blaze Some of the mares bore foals *with the same blaze even after another mating with* another stallion

Another classical instance is Lord Morton's mare This ' nearly purely ' bred Arabian chestnut mare bore a hybrid to a quagga Subsequently she produced two colts by a black Arabian horse These colts were striped on *the legs like the quagga The hair in the mane of these* colts also resembled that of the quagga, being short and stiff Darwin who studied this problem long and thoroughly, in his characteristic way, had no doubt that the quagga had affected the character of the offspring subsequently begot by the black Arabian horse

Dog breeders hold that a thoroughbred bitch which has had pups from a mongrel will not breed true ever again

The tall specimen is a PHENOTYPE In GENOTYPE it may be TT, HOMOZYGOUS for tallness, or Td, HETEROZYGOUS for tallness The TALL plant of the 'pure breed' in the original work of Mendel was TT

The dwarf specimen is also a phenotype In genotype it is dd

Aid to memory

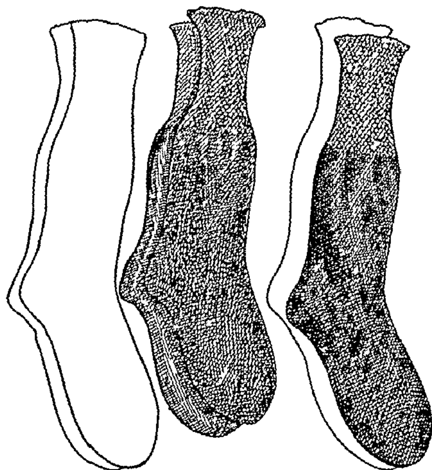


Figure 10 The socks are of the same size and quality The colour varies

A subject A in phenotype may be AA (homozygous) or AO (heterozygous) in genotype Similarly a subject B may be BB or BO in genotype Subjects O and AB are, of course, OO and AB in genotype

Inheritance of Blood Groups in Ten Possible Matings of Four Blood Groups

Of the 4 blood groups there are 6 possible combinations, thus

O	A	B	AB
O × A	A × B		B × AB
O × B	A × AB		
O × AB			

To these must be added 4 pairs made by the same groups, thus —

O × O A × A, B × B, AB × AB

Taken conveniently the pairs are (1) O × O, (2) O × A (3) O × B, (4) O × AB, (5 A × A, (6) A × B, (7) A × AB, (8) B × B, (9) B × AB, (10 AB × AB

The pairs 1, 5 and 8 will yield homozygous products only

In the following diagrams male and female gametes unite like T and d in mendelism

(1) O × O

Male gametes

Female gametes	O	O
O	OO	OO
O	OO	OO

Offspring all O No other group possible All offspring homozygous

Similar occurrences have been observed in other domestic animals and even in birds

The following explanations have been given (1) The spermatozoa may remain alive for a considerable time They do so in European bats and remain simply stored in the uterus for several months In the queen bee they are stored in spermathecæ for years So far as most mammals go this explanation cannot be accepted otherwise offspring will be produced often without a second sire (2) The surplus seminal fluid may be absorbed by the system and affect the ova without fertilizing them This is the INFECTION hypothesis (3) The surplus fluid from the first sire exerts a physiological influence on the constitution of the mother Such an influence is known in botany as XENIA The pollen from the male is capable of affecting the substance of the seed or even the fruit, as distinguished from the embryo itself, formed after a previous fertilization In the plant, however, a 'double fertilization' is seen one for the embryo and the other for the endosperm (4) The mother is influenced through the fœtus during the pregnancy The influence reacts on subsequent offspring The influence may be an endocrine one a strong feature in the fœtus alters an endocrine focus in the mother in the first pregnancy and the altered focus alters the corresponding focus in the fœtus in the subsequent pregnancy Thus is the influence of a past sire exerted on the offspring of the future sire This explanation will not, of course, apply to birds

The quagga like characters have also been explained as a mere ATAVISM reversion to the primitive wild type

MENDELISM IN BLOOD GROUPS

Three Characters

A B and O are three allelomorphs out of which A and B are dominant (like T) and O is recessive (like d) This is Bernstein's hypothesis

(5) A × A

(i) AA × AA

	A	A
A	AA	AA
A	AA	AA

Offspring all A
No other group possible All offspring homozygous

(ii) AO × AO

	A	O
A	AA	AO
O	AA	AO

Offspring A and O
Other groups not possible

(iii) AA × AO

	A	O
A	AA	AO
A	AA	AO

Offspring all A
No other group possible

Appearance of O in the offspring of parents A should be noted

(6) A × B

(i) AA × BB

	B	B
A	AB	AB
A	AB	AB

Offspring all AB
No other group possible

(ii) AA × BO

	B	O
A	AB	AO
A	AB	AO

Offspring A and AB Other groups not possible

(iii) AO × BB

	B	B
A	AB	AB
O	OB	OB

Offspring B and AB Other groups not possible

(iv) AO × BO

	B	O
A	AB	AO
O	OB	OO

Offspring of all groups

(7) A × AB

(i) AA × AB

	A	B
A	AA	AB
A	AA	AB

Offspring A and AB
Other groups not possible

(ii) AO × AB

	A	B
A	AA	AB
O	OA	OB

Offspring A B and AB
O not possible

Impossibility of getting an offspring O from a parent AB should be noted

(2) O × A

(i) OO × AA
(homozygous A)

	A	A
O	OA	OA
O	OA	OA

OA = A
Offspring all A No other group possible

(ii) OO × AO
(heterozygous A)

	A	O
O	OA	OO
O	OA	OO

Offspring O and A Other groups not possible

(3) O × B

(i) OO × BB
(homozygous B)

	B	B
O	OB	OB
O	OB	OB

OB = B
Off pring all B No other group po sible

(ii) OO × BO
(heterozygous B)

	B	O
O	OB	OO
O	OB	OO

Offspring O and B Other groups not possible

(4) O × AB

	A	B
O	OA	OB
O	OA	OB

Offspring A and B Other groups not possible

Impossibility of getting an offspring AB from a parent O and of an offspring O from a parent AB should be noted *Aid to memory Universal Donors and* Universal Recipients cannot be parents and offspring to each other

The combined results of the ten possible matings are given in the table of inheritance of blood characters at the end of this chapter

In summary—(1) A and B cannot appear in an offspring unless they are present in parents (2) A parent O cannot have an offspring AB nor can a parent AB have an offspring O, no matter what the other parent is

Inheritance of Isonins

In the scheme of inheritance of the isogens in the rbc, given above, the isonins in the plasma have not been included The simplest explanation is that both a and b are formed in the body and that the incompatible isonin is absorbed and neutralized at the source by the isogen present there The facts that (*i*) the isonins appear long after the isogens and that (*ii*) the isogens are present in all cells of the body, make this explanation adequate

Another explanation is the linkage between A and b, and B and a (*vide infra*)

Inheritance of Sub groups of A

A_1 is dominant over A (A is dominant over A_3, if the latter can be recognized)

In practice, the sub groups (A_1, A , A_1B and A_2B—also A_3 and A_3B) are usually ignored because of the difficulties of technique The inheritance, however, can be determined by constructing diagrams along the line followed above

Recessive O in A—As has been stated before, the abnormally occurring a serum has been called anti O serum It agglutinates rbc A , because they contain a recessive O, most of them being heterozygous, A O in genotype

This conception is not in accordance with what occurs in the classical examples of Mendelism the recessive character should not have a somatic existence

(8) B × B

(i) BB × BB

	B	B
B	BB	BB
B	BB	BB

Offspring all B No other group possible All offspring homozygous

(ii) BO × BO

	B	O
B	BB	BO
O	OB	OO

Offspring B and O Other groups not possible

(iii) BB × BO

	B	O
B	BB	BO
B	BB	BO

Offspring all B No other group possible

Appearance of O in the offspring of parents B should be noted

(9) B × AB

(i) BB × AB

	A	B
B	BA	BB
B	BA	BB

Offspring B and AB Other groups not possible

(ii) BO × AB

	A	B
B	BA	BB
O	OA	OB

Offspring A B and AB O not possible

Impossibility of getting an offspring O from a parent AB should be noted

(10) AB × AB

	A	B
A	AA	AB
B	BA	BB

Offspring A B and AB O not possible

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This conception is not in accordance with what occurs in the classical examples of Mendelism the recessive character should not have a somatic existence

Further, the question arises why the recessive O in the genotypes AO and BO is not detected by the anti O serum The situation suggests that the dominance of A over O is of a lower order than that of A and B

The somatic existence of the recessive character, similar to that of O in A_2, is also apparent in the Rh types (*see* genetics of Rh)

Dominance of B over A

Some dominance of B over A, especially in A , has been shown (Thomsen, quoted by D Harley, *loc cit*)

This conception, while supporting the lower status of A , is not in accordance with the equality of A and B in the hypothesis of Bernstein

Blood Character C

As stated before, there is evidence for the existence of this character It is associated with A_1 and B but not with A and O

Three Genes p, q, r

If the genes responsible for A, B and *O* be represented by p, q and r respectively then in a particular community they must equal unity (or 100 per cent)

Then union in the community is given by the content of a square on the two sides of which they are represented, thus

	A p	B q	O r
A p	AA p	AB pq	AO pr
B q	BA pq	BB q	BO qr
O r	OA pr	OB qr	OO r^2

The proportion of the four groups in the offspring is as follows —

$$\text{Group O} = r^2$$
$$\text{Group A} = p^2 + 2pr$$
$$\text{Group B} = q^2 + 2qr$$
$$\text{Group AB} = 2pq$$

From this p, q and r are calculated, thus

$$\text{Group O} + \text{Group A} = r^2 + 2pr + p^2 = (r+p)^2$$
$$r + p = \sqrt{\text{group O} + \text{group A}}$$
$$p = \sqrt{\text{group O} + \text{group A}} - r$$
$$p = \sqrt{\text{group O} + \text{group A}} - (1 - p - q)$$
$$p = \sqrt{\text{group O} + \text{group A}} - 1 + p + q$$
$$\text{Zero} = \sqrt{\text{group O} + \text{group A}} - 1 + q$$
$$q = -\sqrt{\text{group O} + \text{group A}} + 1$$
$$q = 1 - \sqrt{\text{group O} + \text{group A}}$$
$$\text{Similarly } p = 1 - \sqrt{\text{group O} + \text{group B}}$$
$$\text{and } r = \sqrt{\text{group O}}$$

The calculation makes it possible to include in the count the recessive O genes in subjects of heterozygous groups A and B

The fact that p, q and r equal unity (or 100 per cent) proves the soundness of the three character hypothesis, 'TRIPLE ALLELOMORPHISM', of Bernstein Apart from this proof there is no merit in extracting the genes from groups while giving the blood groups of a community The four groups are as comparable as the three genes and possess the additional merit of being original observations

Another system of symbols —The three genes have also been designated

$$I^A \text{ (and } I^A \;\; I^A \;\; I^A\text{)} \; I^B \text{ and } I^O \text{ (or } i\text{)}$$

I italicized and large, stands for iso(agglutino)gen in general, and *A*, *B* and *O*, italicized and small in size (capital in form), stand for the isogens in particular Small i may be used for I^O

Further, the question arises why the recessive O in the genotypes AO and BO is not detected by the anti O serum. The situation suggests that the dominance of A over O is of a lower order than that of A and B.

The somatic existence of the recessive character, similar to that of O in A_2, is also apparent in the Rh types (*see* genetics of Rh).

Dominance of B over A

Some dominance of B over A, especially in A_2, has been shown (Thomsen, quoted by D. Harley, *loc. cit.*).

This conception, while supporting the lower status of A_2, is not in accordance with the equality of A and B in the hypothesis of Bernstein.

Blood Character C

As stated before, there is evidence for the existence of this character. It is associated with A_1 and B but not with A_2 and O.

Three Genes: p, q, r

If the genes responsible for A, B and O be represented by p, q and r respectively then in a particular community they must equal unity (or 100 per cent).

Their union in the community is given by the content of a square on the two sides of which they are represented, thus:

	A p	B q	O r
A p	AA p	AB pq	AO pr
B q	BA pq	BB q	BO qr
O r	OA pr	OB qr	OO r^2

mutations Serious somatic upsets are likely to be always associated with non disjunction Similar studies do not appear to have been made in animals

Other Hypotheses

Two independent pairs of Mendelian characters — This is the hypothesis of von Dungern and Hirszfeld, put forward as early as 1910 The genes for the isogens A and B are dominant over those of the isonins a and b The genetic constitution of the four groups is as follows

Group	Two character constitution	Three character constitution
O	aabb	OO
A	AAbb Aabb	AA AO
B	aaBB aaBb	BB BO
AB	AABB AABb AaBB AaBb	AB

According to the hypothesis which was accepted for about 15 years parents O and AB (O × AB) can have off spring O and AB This is not a fact Besides, the frequency of group AB calculated in accordance with the hypothesis differs from the one actually found

[According to this hypothesis

O × AB can have offspring O A B AB
A × AB ,,
B × AB ,,
AB × AB ,,

See Table I serial numbers 7-10]

Complete linkage of two pairs of characters — This is the hypothesis of Furuhata, put forward in 1927 The genes for the isogens A and B and for the isonins a and b are linked completely The gene for A is linked with the gene for b, the gene for B is linked with the gene for a, while the genes for A and B are never linked together The hypothesis also fails in respect of frequency of group AB the calculated figure does not agree with the figure found actually

Exceptions to the Hypothesis of Triple Allelomorphism

The first law of inheritance of blood groups has never been disputed Besides, it holds good according to the other hypotheses also (*vide infra*) The second law provided many exceptions in the beginning The exceptions, however, have decreased steadily with the improvement in and standardization of the techniques of hæmagglutination

A fully proved exception was reported by Haselhorst and Lauer in 1930 and 1931 (quoted by A S Wiener, 1935, BLOOD GROUPS AND BLOOD TRANSFUSIONS, Charles C Thomas, Baltimore, and D Harley 1944, MEDICOLEGAL BLOOD GROUP DETERMINATION, William Heinemann Medical Books Ltd, London) The child of a mother A B was found to be O Tests were repeated with the same results for over two years The child also suffered from gross congenital deformities and was in addition deaf mute and blind It has been suggested that it was subject to a recessive mutation It did not acquire a new character but merely *failed* to develop what was its due A similar unexplained exception was reported by Kessovitch (Wiener, *loc cit*) The possibility of a mutation resulting in acquiring what is not due, however remains for other exceptions that may be reported

Another explanation of such unexpected results may lie in non disjunction of chromosomes in gametogenesis *The two chromosomes bearing the two allelomorphs may* fail to separate (*i e* the reduction division may be in effective) Both the characters then, will be present in one of the gametes and neither in the other The zygotes in both cases (if capable of forming and surviving) will yield unexpected product Such occurrences are often seen in plants and have been held to be responsible for

The following diagrams give all the possible results of their matings

(1) M × M

Male gametes

Female gametes	M	M
M	MM	MM
M	MM	MM

Offspring all M No other type possible

(2) M × N

Male gametes

Female gametes	N	N
M	MN	MN
M	MN	MN

Offspring all MN No other type possible

(3) M × MN

Male gametes

Female gametes	M	N
M	MM	MN
M	MM	MN

Offspring M and MN N not possible

Partial linkage of two pairs of characters —In this hypothesis, put forward by Kirahara and Haku in 1927 and by Baur in 1928, the linkage (of Furuhata) between the genes for the isogens and those for the isonins is partial A crossing over is possible and explains the occasional appearance of children of groups O and AB in the matings of O and AB

The superiority of the three character hypothesis over a two character hypothesis of any kind is established by the equation, $p + q + r = 1$

INHERITANCE OF BLOOD TYPES

Two Dominant Characters of Equal Value

M and N are both dominant and no recessive character (like O in the O A B system) is associated with them in the M N system

A subject M in phenotype is MM in genotype, a subject N in phenotype is NN in genotype and a subject MN in phenotype is, of course, MN in genotype (like the subject AB in the O A B system)

Inheritance of Blood Types in Six Possible Matings of Three Blood Types

Of the 3 blood types there are 3 possible combinations, thus —

M, **N,** **MN**

M × N N × MN

M × MN

To these must be added 3 pairs made by the same types, thus —

M × M N × N MN × MN

Taken conveniently the pairs are (1) M × M (2) M × N, (3) M × MN (4) N × N, (5) N × MN (6) MN × MN

The latest nomenclature in the genes, carrying the M and N characters associated with S, postulates 4 genes L^M, L^{Ms}, L^N and L^{Ns}. The symbol L has been selected in honour of Landsteiner who put blood groups on a scientific basis. Enough work has not yet been done on their inheritance (A S Wiener, *Bulletin of the New York Academy of Medicine loc cit*)

INHERITANCE OF Rh

Rh + and Rh −

If the original division of the Rh state of rbc only be considered the situation is very simple. Rh + state, the character Rh, is dominant over the Rh − state, the character rh.

An Rh + subject may be homozygous, RhRh or heterozygous, Rhrh. The Rh − subject is of course homozygous rhrh.

Inheritance of Rh +/− State in Six Possible Matings of Three Genotypes

Of the 3 genotypes there are 3 possible combinations, thus —

RhRh,	**Rhrh,**	**rhrh**
RhRh × Rhrh		Rhrh × rhrh
RhRh × rhrh		

To these must be added 3 pairs made by the same genotype, thus —

RhRh	×	RhRh
Rhrh	×	Rhrh
rhrh	×	rhrh

Taken conveniently the pairs are (1) RhRh × RhRh, (2) RhRh × Rhrh, (3) RhRh × rhrh, (4) Rhrh ×

(4) N × N

Male gametes

Female gametes	N	N
N	NN	NN
N	NN	NN

Offspring all N No other type possible

(5) N × MN

Male gametes

Female gametes	M	N
N	NM	NN
N	NM	NN

Offspring N and MN M not possible

(6) MN × MN

Male gametes

Female gametes	M	N
M	MM	MN
N	NM	NN

Offspring of all types

The results of matings are also given in the table of inheritance of blood characters at the end of this section

(4) Rhrh × Rhrh

Male gametes

Female gametes	Rh	rh
Rh	RhRh	Rhrh
rh	rhRh	rhrh

Offspring RhRh Rhrh and rhrh (Rh negative)

(5) Rhrh × rhrh

Male gametes

Female gametes	Rh	rh
rh	rhRh	rhrh
rh	rhRh	rhrh

Offspring Rhrh and rhrh

(6) rhrh × rhrh

Male gametes

Female gametes	rh	rh
rh	rhrh	rhrh
rh	rhrh	rhrh

Offspring rhrh

Rhrh, (5) Rhrh × rhrh, (6) rhrh × rhrh The following diagrams give all the possible results of these matings

(1) Rh × RhRh
Male gametes

Female gametes	Rh	Rh
Rh	RhRh	RhRh
Rh	RhRh	RhRh

Offspring RhRh (Rh positive homozygous)

(2) RhRh × Rhrh
Male gametes

Female gametes	Rh	Rh
Rh	RhRh	RhRh
rh	rhRh	rhRh

Offspring RhRh and Rhrh (Rh positive heterozygous)

(3) RhRh × rhrh
Male gametes

Female gametes	Rh	Rh
rh	Rhrh	Rhrh
rh	*rhRh*	*rhRh*

Offspring Rhrh

The genotypes of the Rh phenotypes are as follows —

Phenotypes	Genotypes
rh	rr
Rh′	r′, rh and r′r
Rh″	r″r″ and r″r
Rh′Rh″	r′r″ $r^y r$, $r^y r$ $r^y r''$ and $r^y r^y$
Rh_o	$R^o R^o$ and $R^o r$
Rh_1	$R^1 R^1$ $R^1 r'$ $R^1 r$ $R^1 R^o$ and $R^1 r'$
Rh_2	$R^2 R^2$ $R^2 r''$ $R^2 r$ $R R^o$ and $R^o r''$
$Rh_1 Rh_2$	$R^1 R^2$ $R^1 r''$ R r R r $R^z r'$ $R^z r''$, $R^z r^y$ $R^z R$ $R^z R^1$ $R^z R$ $R^z R^z$, $R^o r^y$ $R^1 r^y$ and $R^2 r^y$

(Adapted from A S Wiener *Bulletin loc cit*)

Allelomorphs in Rh —Unlike the classical example of T and d in Mendelism no character is recessive in Rh It is possible to detect rh in the rbc by St serum It is also possible for the c, d and e to exist alongside C, D and E and be detected by anti c serum, anti d serum and anti e serum respectively How the physical basis of the recessive character in the genes finds expression in the somatic tissue must be explained A similar occurrence is known in respect of A and O

Inheritance of 8 Rh types 1 Wiener's hypothesis —There is a single locus for the Rh alleles on each of a pair of chromosomes On each locus there is one gene The genes on the two loci may be similar (for homozygous product) or dissimilar (for heterozygous product) The eight possible genes which may be found

The results of matings are also given in the table of inheritance of blood characters at the end of this chapter

Rh Types

Rh antigens and genes—The correspondence between the antigen or antigens (occurring together) and the genes, and the frequency of the genes are as follows —

Antigen or antigens	Genes	*Approximate frequency in whites in* U S A (N Y C)
rh	r	38.0
Rh	r	1.4
Rh′	r″	0.5
Rh + Rh″ = Rh_y	r^y	0.01
Rh	R^o	3.2
Rh_1	R^1	40.4
Rh	R^2	16.4
Ph_1 + Rh = Rh	P	0.1

(Abstracted and adapted from A S Wiener 1949 *Bulletin of the New York Academy of Medicine* **25** 255–260)

Rh phenotypes and genotypes—The determination of the 8 Rh types with 3 antisera has been described in detail before The types so determined are phenotypes

(They correspond, in blood groups, to the phenotypes A and B which may be in genotype

AA or AO and

BB or BO respectively)

The genotypes of the Rh phenotypes are as follows —

Phenotypes	Genotypes
rh	rr
Rh'	r', rh' and r'r
Rh''	r''r'' and r''r
Rh'Rh''	$r'r''$, r^yr, r^yr', r^yr'' and r^yr^y
Rh_0	R^oR^o and R^or
Rh_1	R^1R^1, R^1r', R^1r, R^1R^o and R r
Rh_2	R^2R^2, R^or'', R r, R R and R^or''
Rh_1Rh_2	R^1R^o, R^1r'', R r, R^zr, R^zr', R^zr'', R^zr^y, R^zR^o, R^zR^1, R^zR^2, R^zR^z, R^or^y, R^1r^y and R^or^y

(Adapted from A S Wiener *Bulletin loc cit*)

Allelomorphs in Rh —Unlike the classical example of T and d in Mendelism no character is recessive in Rh It is possible to detect rh in the tube by St serum It is also possible for the c, d and e to exist alongside C, D and E and be detected by anti c serum, anti d serum and anti e serum respectively How the physical basis of the recessive character in the genes finds expression in the *somatic tissue must be explained A similar* occurrence is known in respect of A and O

Inheritance of 8 Rh types 1 Wiener's hypothesis —There is a single locus for the Rh alleles on each of a pair of chromosomes On each locus there is one gene The genes on the two loci may be similar (for homozygous product) or dissimilar (for heterozygous product) The eight possible genes which may be found

on the loci are r, r′, r″, r^y, R^o, R^1, R, and R^z There are 36 possible combinations of the genes, as given in the table of inheritance at the end of this chapter

Information on the inheritance of Rh_0 and its combinations is not yet available

2 **Fisher's hypothesis**—There are 3 pairs of allelic genes occupying 3 adjacent loci on each of a pair of chromosomes At one pair of loci occur the combinations CC, Cc or cc, at the second pair occur DD, Dd or dd, and at the third pair occur EE, Ee or ee

Additional antigens have been discovered One of them is C^w at the first locus and another at the second locus Information on their inheritance is not yet available

A similar hypothesis was really considered by Wiener first and discarded in favour of his present hypothesis (A S Wiener, 1949, *Brit Med Journ*, i, 404)

TWINS

The ordinary fraternal twins are biovular or dizygous Their blood characters are like those of brothers and sisters not born simultaneously

The identical twins are uniovular or monozygous Their blood characters must be identical, as has been established in the cases of O A B (including the subgroups of A) and M N Incidentally, the identical twins represent the asexual reproduction in man, growing from asexual reproduction in the sexually produced zygote

SUPERFECUNDATION

The existence of this admittedly rare occurrence can be established by twins, triplets and their blood groups When a wife O of a husband A gives birth to an infant A and infant B the second father must be B Adultery with a subject AB must, however be excluded Triplets O, A and B would definitely establish two fathers

TABLES OF INHERITANCE OF BLOOD CHARACTERS

Table I

Blood groups in parents and children

Serial number	Parents	Children possible	Children impossible
1	O × O	O	A B AB
2	O × A	O A	B AB
3	O × B	O B	A AB
4	A × A	O A	B AB
5	A × B	O A B AB	
6	B × B	O B	A AB
7	O × AB	A B	O AB
8	A × AB	A B AB	O
9	B × AB	A B AB	O
10	AB × AB	A B AB	O

Table II

Blood types in parents and children

Serial number	Parents	*Children possible*	*Children impossible*
1	M × M	M	MN N
2	M × MN	M MN	N
3	M × N	MN	M N
4	MN × MN	M MN N	
5	MN × N	MN N	M
6	N × N	N	M MN

Table III

Rh +/− states in parents and children

Serial number	Parents	Children possible	*Children impossible*
1	Rh + × Rh +	Rh + Rh −	
2	Rh + × Rh −	Rh + Rh −	
3	Rh − × Rh −	Rh −	Rh +

TABLE IV

Eight Rh types in parents and children

Serial number	Parents	If child belongs to one of following type parentage is possible	If child belongs to one of following types parentage is excluded	If child belongs to one of following types parentage is highly improbable
1	rh × rh	rh	Rh Rh Rh Rh_2 Rh Rh Rh″ and Rh Rh″	
2	rh × Rh	rh and Rh	Rh Rh_2 Rh Rh Rh_0 Rh″ and Rh Rh	
3	rh × Rh	rh and Rh″	Rh Rh Rh Rh Rh Rh and Rh Rh″	
4	rh × Rh Rh	Rh and Rh	Rh Rh Rh Rh and Rh	Rh Rh and rh
5	rh × Rh	rh and Rh	Rh Rh Rh_1Rh Rh Rh and Rh Rh″	
6	rh × Rh	Rh Rh Rh_0 and rh	Rh_2 Rh Rh Rh and Rh Rh″	
7	rh × Rh	Rh Rh Rh and rh	Rh Rh Rh Rh and Rh Rh	
8	rh × Rh Rh	Rh Rh_2 Rh and Rh	/	Rh Rh Rh Rh Rh and rh
9	Rh × Rh	rh and Rh	Rh Rh Rh Rh Rh Rh and Rh Rh	
10	Rh × Rh	Rh Rh Rh Rh and rh	Rh Rh Rh Rh and Rh	
11	Rh × Rh Rh	Rh Rh″ and Rh Rh	Rh Rh Rh Rh and Rh	rb
12	Rh × Rh	Rh Rh Rh and rh	Rh Rh″ Rh Rh and Rh Rh	
13	Rh × Rh	Rh Rh Rh and rh	Rh Rh″ Rh Rh and Rh′Rh	

TABLE IV—*contd*

Serial number	Parents	If child belongs to one of following types parentage is possible	If child belongs to one of following types parentage is excluded	If child belongs to one of following types parentage is highly improbable
14	Rh × Rh_2	Rh Rh_2 Rh Rh″ Rh Rh_2 Rh Rh″ Rh_0 and rh		
15	Rh × Rh_1Rh_2	Rh Rh_2 Rh Rh″ Rh_1Rh_2 and Rh′Rh″		Rh_0 and rh
16	Rh″ × Rh″	Rh″ and rh	Rh_1 Rh_2 Rh_1Rh_2 Rh_0 Rh and Rh′Rh″	
17	Rh″ × Rh′Rh″	Rh Rh″ and Rh Rh″	Rh_1 Rh_2 Rh_1Rh_2 and Rh_0	rh
18	Rh × Rh	Rh_2 Rh″ Rh_0 and rh	Rh Rh Rh_1Rh_2 and Rh′Rh	
19	Rh″ × Rh_1	Rh Rh_2 Rh Rh″ Rh_1Rh_2 Rh Rh , Rh_0 and rh		
20	Rh″ × Rh_2	Rh Rh Rh_0 and rh	Rh Rh Rh_1Rh_2 and Rh Rh	
21	Rh × Rh Rh_2	Rh Rh Rh Rh Rh Rh_2 and Rh Rh		Rh_0 and rh
22	Rh′Rh″ × Rh Rh	Rh Rh and Rh′Rh	Rh_1 Rh_2 Rh_1Rh_2 and Rh	rh
23	Rh Rh × Rh	Rh_1 Rh Rh_2 and Rh		Rh_1Rh_2 Rh Rh″ Rh_0 and rh
24	Rh Rh″ × Rh	Rh_1 Rh Rh_2 Rh Rh_1Rh_2 and Rh′Rh		Rh_0 and rh
25	Rh Rh′ × Rh	Rh Rh Rh Rh Rh Rh_2 and Rh Rh		Rh_0 and rh
26	Rh Rh″ × Rh_1Rh_2	Rh Rh Rh_2 Rh Rh Rh_2 and Rh Rh		Rh_0 and rh
27	Rh_0 × Rh_0	Rh_0 and rh	Rh Rh_2 Rh_1Rh_2 Rh Rh and Rh Rh	
28	Rh_0 × Rh_1	Rh_1 Rh , Rh_0 and rh	Rh_2 Rh Rh_1Rh_2 and Rh Rh	
29	Rh_0 × Rh	Rh_2 Rh Rh_0 and rh	Rh_1 Rh Rh_1Rh_2 and Rh′Rh	

TABLE IV—*concld*

Serial number	Parents	If child belongs to one of following types parentage is possible	If child belongs to one of following types parentage is excluded	If child belongs to one of following types parentage is highly improbable
30	Rh_0 × Rh Rh	Rh Rh_2 Rh and Rh		Rh Rh_2 Rh Rh″ Rh_0 and rh
31	*Rh* × *Rh*	*Rh* *Rh* *Rh* and rh	*Rh* *Rh* *Rh Rh* and Rh′Rh″	
32	Rh_1 × Rh_2	Rh Rh_2 Rh Rh Rh Rh_2 Rh Rh Rh and rh		
33	Rh × Rh Rh	Rh_1 Rh_2 Rh Rh Rh Rh_2 and Rh′Rh		Rh and rh
34	Rh × Rh_2	Rh Rh Rh and rh	Rh Rh Rh Rh and Rh′Rh″	
35	Rh_2 × Rh Rh	Rh Rb Rh Rh Rh Rh and Rh Rh		Rh and rh
36	Rh Rh_2 × Rh Rh	Rh Rb Rh Rh Rh Rh_2 and Rh′Rh		Rh and rh

From Wiener (*Jour Lab and Clin Med* **31** 575-583 May 1946) *with permission*

Rh Rh and Rh Rh are now known as rh rh and rh rh″ respectively The present author sees no harm in retaining the original symbols

VIII

FORENSIC APPLICATION OF BLOOD CHARACTERS

BLOOD STAINS ON CLOTHES AND OTHER EFFECTS

An accused with his clothes or other effects stained with blood which is not of his own group is in possession of some one else's blood He may be called upon to explain how he obtained it The stains can be tested for the group of the blood

For the test of the stain valuable articles need not be damaged The stain can be scraped, removed on a wet filter paper or washed off For washing the selected surface is ringed round with hard paraffin (melted but not very hot) A capillary teat pipette floods it with saline and sucks back the saline, repeatedly The saline containing blood is dried in a desiccator A fabric is treated similarly after stiffening its back with hard paraffin Blood from joints of valuable weapons is also collected in the same way, by means of a pipette ejecting and sucking back saline

The stains or dried washings are tested by absorption with a serum ab (from a subject O) as detailed previously (p 22)

So far only the characters O A B have been used in testing stains for forensic purposes

Blood group of the deceased in a case of murder — Usually a requisition is received for a comparison between the stains caused by the victim's blood and those found on the accused, weapons or place of occurrence These latter may be insufficient or unsuitable for grouping tests and a comparison may not be possible In that case the group of the deceased only may be determined A new enquiry later may find new accused persons,

TABLE IV—*concld*

Serial number	Parents	If child belongs to one of following type parentage is possible	If child belongs to one of following types parentage is excluded	If child belongs *to one of* following types parentage is highly improbable
30	Rh_0 × Rh Rh	Rh Rh Rh and Rh		Rh_1Rh Rh′Rh″ Rh_0 and rh
31	Rh_1 × Rh_1	Rh Rh Rh and rh	Rh Rh″ Rh Rh_2 and Rh′Rh″	
32	Rh × Rh	Rh Rh Rh Rh Rh_1Rh Rh Rh Rh and rh		
33	Rh × Rh Rh_2	Rh_1 Rh Rh Rh Rh Rh and Rh Rh″		Rh_0 and rh
34	Rh_2 × Rh	Rh Rh Rh and rh	Rh Rh Rh Rh and Rh Rh″	
35	Rh × Rh Rh	Rh Rh_2 Rh Rh″ Rh Rh_2 and Rh Rh		Rh and rh
36	Rh Rh × Rh Rh	Rh Rh_2 Rh Rh″ Rh Rh and Rh′Rh		Rh and rh

From Wiener (*Jour Lab and Clin Med* **31** 575–583 May 1946) with permission

Rh Rh and Rh Rh are now known as rh rh″ and rh rh″ respectively The present author sees no harm in retaining the original symbols

Recipients cannot be parents and offspring to each other) The reason lies in the genetics of subjects O and AB

A_1 is dominant over A_2 The sub groups of A are ignored in forensic medicine by most workers because of technical difficulties The new blood character C when studied fully will differentiate them easily There are two laws of inheritance (1) No child can belong to sub-group A_1 or sub group A_1B unless one or both parents belong to one of these sub groups For example, two parents both of sub group A_2 cannot have a child of sub group A_1 (2) Parents of sub groups A_1B cannot have children of sub group A_2, and parents of sub group A_2 cannot have children of sub group A_1B

A_1 is dominant over A_2

M-N

The table of inheritance of M-N (p 129) gives the possible combinations of parents and offspring Two rules emerge (1) M and N cannot appear in the offspring unless they are present in one or both parents (2) A parent M cannot have an offspring N, nor can a parent N have an offspring M

O A B and M-N are not interrelated Usually the former system is determined first and the latter later But either of them excludes parenthood with equal justification The fact that M-N system was discovered later does not make it more up to date and therefore more reliable

As has been stated before, under the M N system, the discovery of Rh has made the preparation of the testing fluids more difficult On the other hand, the newly discovered antigen S, found associated with M and N, has increased the number of types and consequently their forensic value Enough work, however, has not yet been done on their inheritance (A S Wiener, 1949, *Bulletin of the New York Academy of Medicine, loc cit*)

weapons and places of occurrence The deceased's blood may then not be available

Presumption of the existence of body fluids—Saliva may be presumed to be present on cigarette ends and nasal secretion on handkerchiefs The latter may also be stained with sputum Even postal stamps affixed to a letter may carry saliva, if they were licked An unlicked stamp of the same batch must be available as a control

Indication versus evidence—From very small quantities there may be derived an *indication* for the purpose of investigation, instead of a *deposition* for the court

BLOOD CHARACTERS IN EXCLUDING PATERNITY AND MATERNITY

Disputes in paternity and maternity arise (1) When a child is alleged to be supposititious This appears to be the commonest cause in India (2) When a man is averred by a woman to be father of her child This is the commonest cause in Europe (3) When a husband avers that he is not the father of his wife's child (4) When children are suspected to have been changed in the maternity ward of a hospital

O-A-B

The table of inheritance of O A B (p 129) gives the possible combinations of parents and offspring Two rules emerge (1) Characters A and B cannot appear in the offspring unless they are present in one or both parents It might appear that this rule does not apply to the character O which can appear in the offspring without being the blood group of either parent This is not so In such cases O is present in a recessive form in both parents (2) A parent O cannot have an offspring AB nor can a parent AB have an offspring O (Aid to Memory Universal Donors and Universal

P + and P −

The P + and P − types can also be used for forensic purposes when the testing serum becomes available From the table in the chapter on anthropology the character P appears to be distributed a little more evenly in India than elsewhere (The number of subjects tested, however, is insufficient statistically)

Other hæmogens have been mentioned previously (pp 81, 82) Their forensic value is not yet proved

POSITIVE AND NEGATIVE FINDINGS

Stains

A requisition for determining the *identity* of blood in two or more stains cannot obviously be complied with All that can be determined is whether the bloods in the stains belong to the same group

The determination of the lack of identity, on the other hand, is an easy matter If the stains do not belong to the same group they could not have been caused by an identical blood Even when the group of one of them is in doubt the lack of identity can be established

Example

(*i*) The group of blood on an exhibit is B

(*ii*) The group of blood on another exhibit in the [illegible]me case is in doubt It is probably O But it is not B [illegible]The lack of identity is established

Parentage

[illegible]ve finding is definite while the positive [illegible] [illegible]possibility only It can be stated defin[illegible] [illegible]om is *not* the son of Mr Smith It canno[illegible] definitely that he *is* the son of Mr Brown [illegible] *be* the son of Mr Brown is all that can be st[illegible] the positive side The same remarks apply to M[illegible] Smith and Mrs Brown regarding motherhood

Parentage excluded by the examination of one-parent only—This is possible when the second law of

Rh

A simple plan of inheritance—Such a plan could be considered useful only seven years ago and is shown in the table of inheritance of the Rh + or Rh − state (p 129) Three rules emerge (1) When both parents are Rh +, the offsprings can be Rh + or Rh − (2) When one parent is Rh + and the other Rh −, the offspring can be Rh + or Rh − (3) When both parents are Rh −, the offspring must be Rh −

A complicated plan of inheritance—A table much larger than those on O A B or M-N, as prepared by Wiener on the inheritance of the various types of Rh, is also included in the list of tables Two rules emerge (1) The antigens Rh_0, Rh′ and Rh cannot appear in the blood of a child unless present in the blood of one or both parents (2) When either parent belongs to type Rh_1Rh or Rh′Rh″, no child of type rh or Rh_0 can occur For the same reason parents of type rh or Rh_0 cannot have children of type Rh_1Rh or Rh′Rh″

Rh blood characters, in their present stage in study, may not be used as evidence against parentage Apart from the difficulties of the serological technique and genetics, frequent modifications of the nomenclature, such as have been made during the last five years, are likely to create a bias against them in a court of law Further, the possibility of ‘ rare but valid exceptions to the second rule ’ (Wiener, *loc cit*, abstract in *Indian Med Gaz* 1947, **82**, 165) remains

Hr

The study of this character is too incomplete at present to be used for forensic purposes

CDE

The system was based on prophecies all of which have now been fulfilled (subject to remarks on p 62) It has the advantage of combining both Rh and Hr Its forensic utility is little at present

The fact that O, A, B and AB are more evenly distributed in India than in Europe and America, and *therefore give more information in forensic matters,* has not yet been fully realized

More detailed tables will be found at the end of the chapter on anthropology

DIRECTIONS FOR GETTING BLOOD TESTED

When Fresh Blood can be given

The persons concerned visit a serological laboratory with their photographs The serologist will test the blood and issue the report, keeping his record in the usual register as well as on the photographs

Baby's blood—Isonin is often absent It is usually present when the baby inherits its mother's blood group Even isogen may be absent or very weak, the blood, therefore, should be tested at monthly intervals for a year or so In the famous exceptions to Bernstein's theory (p 118) the blood was examined repeatedly over a period of two years

When Blood is sent to a Distant Laboratory

The author's laboratory requires the following (1) About 0 25 cc of blood dried as a stain on chemically pure filter paper, not in the sun An unstained part of the filter paper should also be available as a control (2) About 0 5 cc of clear serum in an ampoule This is taken from a test tube in which about 3 cc of blood have been put and allowed to clot *under sterile conditions,* without disturbing the tube (3) About 0 5 cc of serum turbid with rbc in an ampoule This is taken after shaking gently the contents of the tube, after the clear serum has been removed The specimens are taken by a responsible medical man (preferably an official) and sent in a sealed and registered parcel The impression of the seal is sent in another registered parcel

inheritance of blood groups and blood types applies A parent O cannot have an offspring AB, no matter what the group of the other parent is nor can a parent AB have an offspring O, no matter what the group of the other parent is Similarly, a parent M cannot have an offspring N, no matter what the type of the other parent is nor can a parent N have an offspring M, no matter what the type of the other parent is √

DISTRIBUTION OF BLOOD GROUPS, BLOOD TYPES AND RHESUS GROUPS IN INDIA

The various blood characters have a distinctive distribution all over the world The following percentages are given for comparison —

	O	A	B	AB	
Indian Calcutta	35 03	22.5	34 9	7 9	(S D S Greval and S N Chandra 1940 *Ind Jour Med Res* **27** 1109)
English London	45 9	42 0	9.3	2 7	(G L Taylor and F W Ikin 1939 *Brit Med Journ* i p 1097)
	M	MN	N		
Indians Calcutta	42 7	46 7	10		(S D S Greval S N Chandra and I S F Woodhead 1939 *Ind Jour Med Res* **26** 1041)
English	32.5	48.5	19.5		(D Harley 1936 *Brit Jour Exper Path* **17** 143)
	Rh +	Rh —*			
Indian Calcutta	90 0	10 0			(S D S Greval and A B Roy Chowdhury 1943 *Journ Ind Med Assoc* **13** 65)
White Americans	85 0	15 0			(K Landsteiner and A S Wiener 1940 *Proc Soc Exper Biol and Med* **43** 223)

* With animal serum including Rh and Rh″

(2) In 1939 a provision was made for the evidence of blood tests in the Bastardy (Blood Test) Bill introduced in the House of Lords (*B M J*, Editorial, 1939, *ii*, 453)

The bill received its second reading and was ordered to be sent to a select committee The committee made a few amendments Although the bill received general approval it met with opposition also The opposition was based on three different considerations (*i*) The cost The bill, it was said, would not work in practice because of the inability to pay of the poor who were mostly concerned in such cases (*ii*) No need for scientific evidence Intercourse at the material time should, it was suggested, render a man liable to pay for the maintenance of the child The man who might have been *the father must pay* (*iii*) *Lack of perfection in science* Science, it was feared, was not yet sufficiently advanced to justify the use of blood tests for determining paternity in courts in England The bill which was to come into *operation as an act on the 1st day of January, 1940,* lapsed at the end of 1939 with the prorogation of the Parliament [D Hailey, MEDICOLEGAL BLOOD GROUP DETERMINATION, 1944, London, William *Heinemann* (Medical Books) *Ltd*]

(3) In 1941 a case was decided in India on the evidence of blood groups The facts of the case are as follows

Kengappa and his wife Chennamma complained that their 4 year old son had been kidnapped a year previously by Syed Fakir and his wife Fathima Bee who, however, stated that the boy was their own They pointed to the fact that Fathima Bee was feeding the child on the breast

The blood test showed the child and Fathima to belong to group A Syed Fakir, Kengappa and Chennamma belonged to group O

The magistrate decided the case entirely on the evidence of blood test Indeed no other evidence of any

The group is determined from the stain by absorption The serum corroborates the finding The rbc preserved in the turbid serum (if all has gone well with sterility) also corroborate the findings If the groups exclude the parentage a report stating the facts and conclusions is issued If the groups do not exclude the parentage determination of types is suggested For this purpose fresh blood is required

Opinion is withheld if a 'defective group' [in which an iso(hæmaggluti)nin which could be present is absent] or an anomalous reaction complicates an incompatible combination of groups (not in accordance with the tables) Fresh blood is required for repeating the tests

Blood can be flown in a fresh condition It is taken in a sterile phial which is corked securely with string and left in a refrigerator at 4-8°C A wide mouthed thermos flask loosely stuffed with cotton wool and prepared to receive the phial is also left open in the refrigerator After an hour or so the phial is quickly placed in the flask, and the latter closed and taken to the airport for despatch to the laboratory The laboratory receives the sample by special arrangements, if necessary, and undertakes the examination at once From the fresh clot rbc are washed out with saline The serum is also available for confirmatory test (and other tests of clinical importance)

JUDICIAL ASSESSMENT OF THE EVIDENCE ON BLOOD GROUPS AND BLOOD TYPES

The courts keep the evidence of blood groups and blood types on the same footing as any other evidence (*B M J*, Editorial, 1944, *i*, 134)

The sequence of recent relevant events is as follows —

(1) In 1938 a case was decided in England on the evidence of blood types (*B M J*, Editorial, 1938, *i*, 1135)

instead of, as at present, keeping it on the same footing as other evidence '

Commenting on similar events in general, another expert complains 'In uncontested divorce actions, an exclusion of paternity by the blood tests is usually accepted without question by the court, but when the action is contested, unfortunately, there are still some judges who believe the obviously false testimony of the mother even though the objective findings of the blood tests prove that the husband is not the father of the child in question' (A S Wiener, 1944, *Exp Med and Surg*, **2**, 44)

Evidently there exists a very serious difference of opinion between experts, which needs explanation The authority of the court is undeniable

A danger of inverting the evidential value of the blood tests also exists under the circumstances complained of The negative finding that Master Tom *cannot be* the son of Mr Smith, which is fully guaranteed by the known laws of genetics, is likely to be ignored if the mother's looks and voice impress the judge in her favour This may be called a rejection of positive evidence On the other hand, a positive finding of the possibility only, that Master Tom *can be* the son of Mr Brown, is likely to be unduly stressed by the defence or even by the impressed judge This would be an acceptance of what is not evidence at all but only a possibility

ARTIFICIAL INSEMINATION FROM DONOR IN CASE OF Rh INCOMPATIBILITY IN MARRIAGE

The offspring of the woman inseminated from a man other than the husband will of course not be legitimate The persons responsible for the registration of the birth will be making an incorrect statement Those who know about the insemination will be making a false statement which they know to be false

value was forthcoming (from a report by G P Charlewood, Captain, I M S, Civil Surgeon, Coorg, 1941, *Indian Med Gaz*, **76**, 482)

(4) In 1944 the evidence of blood types was not considered reliable in a case in England (*B M J*, Editorial, 1944, *i*, 134) The judge 'did not, having heard and seen the wife, think she was of a type that commits adultery'

(5) In 1947 the evidence of blood groups was not accepted against a claim of paternity brought against Charles Chaplain in the U S A Examination of the bloods of Charles Chaplain (said to be the father), Joan Berry (the mother) and Carol Ann Berry (the child) gave the following results

	Group (O A B)	Type (M N)
Charles Chaplain	O	MN
Joan Berry	A	N
Carol Ann Berry	B	N

(*J Amer Med Assoc*, 1947, **133**, 498, *Indian Med Gaz* 1947, **82**, 763)

In a similar case one should be on one's guard against questions like the following

Q 1 Do you admit, Doctor, that O A B is an old system and M-N a later development?

Q 2 Do you also admit that this later development is in agreement with the claim while the old system is not?

It must be explained to the court that the two systems are not linked in any way and that the agreement with the second system does not mean that a lack of agreement with the first can be ignored

Commenting on the event (4) the expert writing the editorial referred to above observes with satisfaction 'Few who heard the decision doubted its wisdom It would be an evil day for our courts if they ever felt obliged to elevate scientific evidence into a class by itself,

acquisition of a character but only a *failure* of development of an expected character Such a failure might result from non genetical causes which produce deformities due to failure of normal development

Comparison between the first and second rule of inheritance of O-A-B—It has been stated by European and American workers that exceptions to the first rule were never or hardly ever reported The reason might well be that the majority of subjects under their observation belong to group O and group A, a fact which rather limits the chances of detecting impossible combinations, because O can be derived from A In India where O, A and B are more evenly distributed and the detection of such combinations is easier exceptions may be found

Another reason why exceptions to the second rule were reported was that the occurrence now considered impossible was once upon a time considered possible With the proper understanding of the technique of grouping blood, they have certainly become rare This argument is in favour of the present genetics of blood groups

Telegony as a defence—The child of a previously married woman may resemble the previous husband The resemblance may also cover blood characters In such an unusual proposition the only point deserving a consideration would be the belief held by Darwin in the quagga episode (p 109)

AMPLIFICATION OF SOME GENETIC CONSIDERATIONS

Mutations.—Anthropologists freely admit the possibility of the occurrence of MUTATIONS, sudden and spontaneous changes, in the blood characters, in ethnological groups, when their findings do not fit in with mere mixing of blood between neighbours. Statisticians do not consider any figures on blood characters complete without bringing in mutations. The mutations are, of course, extremely rare events while the medicolegal cases which might be defended by presuming their occurrence are by no means rare. The connection between them must, therefore, be slender.

Non-disjunction of chromosomes.—As has been mentioned before, at the time of division, in a gametocyte, the opposite members of a pair of chromosomes may fail to separate (*see* fig. 9 on p. 105). One gamete then will have an excess and the other a deficit. If such gametes do not fail to form zygotes, and the zygotes do not fail to develop, unexpected blood characters may be encountered in the offspring. A mother AB, for instance, may form a gamete without A or B (one gamete having both A and B and the other none) which after fertilization by a father O may develop into a zygote forming an offspring O. Or a mother AB may have an offspring AB (from a gamete having both A and B) from a husband O.

Such accidents in the division of the gametocytes are known in plant physiology. They, however, cause upsets in the specimens produced by such zygotes. The *deformed child reported by Haselhorst and Lauer* (p. 118) might well have been formed from a zygote resulting from a gamete in which non-disjunction had occurred. The defence of non-disjunction in a normal child, on the other hand, might not be considered strong.

Abnormality due to a failure, not an acquisition.—In the aforesaid deformed child, there was no unexpected

IX

ANTHROPOLOGICAL APPLICATION OF BLOOD CHARACTERS

Blood Group (O-A-B System)

The characters O, A and B are found in all races of u in different proportions. In Europe O and A are ut equal and high while B is low. In India B and O about equal while A is a trifle lower than both. ongst the Eskimos O is the predominating character.

It has been surmised that O is the original blood racter and that A and B arose as mutations, A in ope and B in Asia. The various proportions in ous countries and amongst various racial groups resulted from mixing of races. The author has d the system O A B for this reason; usually it is called A B O system.

The surmise, however, leaves much room for doubt. 1. Did the mutations occur after the *Homo sapiens* had replaced the previous *Homo* and sub *Homo* species? 2. Did he actually replace all *Homo* and sub *Homo* species? 3. Was the replacement (if it occurred) effected by an annihilation or assimilation of the previous population? 4. The characters O, A and B are found in apes and even in other animals, and must have been present in *Homo*, sub *Homo* and sub *sapiens* species always.

The same mutations might have occurred in two unrelated localities at the same time or at different times.

The human race itself might have originated in several localities with a difference in blood groups. The

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European type) They were isolated before the mutation B occurred in Asia

Formulæ and indices have been worked out for races They all depend on the relative frequencies of A and B Symbols p, q and r also have been used in giving the racial distribution of blood groups As has been pointed out before there is no particular merit in working with them

Concerning the technique 1 The measurements —The measurement of volumes cannot be sacrificed to any expediency in field work in anthropology Nor can the period of observation of hæmagglutination be shortened

The calibrated finger pipette, with an inner coating of sodium citrate to keep blood from coagulating, delivers 50 drops to 1 cc and thus measures in 1 drop 1/50 cc One such drop of whole blood added to ½ cc of saline = ½ drop of rbc (only half of whole blood consists of rbc) in 25 drops of saline = 1 drop in 50 drops = 2 drops in 100 drops = 2 per cent suspension

[Actually there are inaccuracies in the procedures (1) Blood is more viscous than saline and its drop bigger (2) As measured, ½ drop of rbc in 25 drops of saline = ½ drop in 26 drops This inaccuracy can be avoided by removing from the ½ cc of saline 1 standard drop before adding the whole blood The error is, however, compensatory it adds more saline than is required for a drop of blood which is actually bigger than it is taken to be Whatever the error may be it will be negligible and remain of the same order for field work]

Washing of the rbc free of plasma is not necessary in field work as it is in clinical work

Nowadays when air mail is available the question of taking blood in the field and sending it quickly to a central laboratory should be considered For collecting samples 1 oz sterile phials and corks are the best One drop of blood allowed to drop by itself from the finger into 24 drops of saline (1½ cc) will provide the 2 per cent

polytypicity of the race is suggestive of such an occurrence

Certain types of blood group distribution—The following types of distribution correlated with racial types of man are recognized —

1 **The European type**—This has been given already A is high while B is low A decreases from north west to south east in Europe

2 **The Intermediate type**—A and B are about equal

3 **The Hunan type**—The name is derived from the province of Hunan in South China A is exceptionally high and B moderately low It includes the Japanese, Koreans nearest Japan, South Chinese, Hungarians, Poles, Ukranians and Egyptians, suggesting the possibility of Mongol relationship

4 **The Indo Manchurian type**—B is high and A is rather low The highest frequency of B is reached in India and Asia It decreases westwards reaching the lowest mark in Europe The mutation it is suggested, occurred in Asia after the American Indian stock had separated

The Gypsies and Ainu (of Japan) are included in this type

5 **The Africo Malaysian type**—Both A and B are moderately developed The type includes the Malayans and Africans

6 **The Pacific-American type**—O is high while A and B are low Full blooded American Indians are probably all O *They were it is suggested, isolated* from Europe and Asia before the mutations A and B occurred

7 **The Australian type (Australian aborigines)**—O and A are high while B is very low (like the

groups A and B evolved from O or has the group O evolved from the group AB by two steps ?

Blood groups in India.—A table at the end of this chapter gives the findings of workers in India. Non-Indian figures are included for comparison.

The following conclusions emerge :—

(*i*) The European figures for Calcutta are quite unlike the European figures from elsewhere. They approximate to the Anglo-Indian figures. Inclusion of the Anglo-Indians with the domiciled Europeans and of the latter with the European mercantile community resident in Calcutta is likely. The Presidency General Hospital, Calcutta, from the in-patient department of which the material was obtained, is essentially a hospital for the European population of Calcutta. A few non-European patients are also admitted. They have been excluded from the table. Jews and Armenians (living like Europeans) have also been excluded.

(*ii*) The Anglo-Indian figures show a distinct rise in B but remain of the European type inasmuch as A is appreciably higher than B. All the subjects tested have, on the whole, approximated to the European type in stature, build, features, and complexion. In this respect a selection in favour of the type has operated. So far as the author is aware no other figures of this community are available.

(*iii*) The figures for Vaidyas (also spelt Baidyas) though derived from a small number are striking. O predominates for no obvious reason. This community though not large is widespread. Inbreeding is excluded by the general Hindu laws of marriage. On the contrary according to Risley (H. H., 1891, THE TRIBES AND CASTES OF BENGAL, Calcutta, Bengal Secretariat Press, p. 47), Porter (A. E., 1933, ' CENSUS OF INDIA,

suspension The corked phials can be carried in a wide mouthed thermos which (with the contents) has been kept uncorked in an ice box for an hour and then corked

2 **The moist chamber**—The slides must be kept in a moist chamber and the reaction observed for at least 15 minutes Ordinarily the slides begin to dry up within a few minutes

3 **Errors resulting from faulty technique**—(1) Coagulated blood may appear like agglutinated blood Whole blood mixed with testing serum is likely to produce such an appearance at times (2) An excess of rbc and a deficiency of testing serum, when measurements are not standardized, may prevent the development of hæmagglutination, specially when the serum is not strong All groups may, then, be returned as O (3) Rbc 'slow' A take a long time to react and may be returned as O unless watched in a moist chamber (4) Some rbc AB may be returned as B because of the slow A It appears that while all A are slow in reacting all slow A are not A

Subtle mathematical formulæ applied to the results later will not compensate for these errors

Concerning a change of plan—The anthropologists may also study blood groups (and other blood characters) of non aboriginal population in Blood Banks which now exist in many centres in India The donors of blood may then be studied at leisure in respect of other anthropological features also The subjects will be coming from a thoroughly mixed population and will be free from the influence of inbreeding on blood groups, which is likely to be encountered in examining small communities living in isolation more or less The author advocated such a plan many years ago

Biological problems—Do new types arise because of the appearance of a new character or because of the accentuation of old potentialities consequent on the disappearance of an old character—zoogenesis ? Have the

The work done on A_2 (and also A_3) has so far been far from full, particularly in India. The new antigen C may make the differentiation between the sub groups of A easier than it has been and thus open up almost a new field for investigation.

Blood Types (M-N System)

The blood types have also a characteristic racial distribution like the blood groups. In the table of blood characters, at the end of this chapter, are given the Indian figures and also non Indian figures for comparison.

The work done on blood types, dating from 1927, is not so extensive as that on blood groups. Besides, the preparation of the testing fluid is rather difficult. Further, the work has been pushed in the background by Rh types. Furthermore, the work must be repeated because of the rhesus grouping. The O cells used in preparing the testing fluid must be of the same Rh type.

The latest nomenclature in the genes, carrying the M and N characters associated with S, postulates 4 genes L^M, L^{MS}, L^N, and L^{NS}. (The symbol L has been selected in honour of Landsteiner who put blood groups on a scientific basis.) Enough work has not yet been done on their racial distribution.

The foregoing considerations are enough to keep the serologically inclined anthropologists busy for some time.

Biological problems.—If a new type is evolved by the disappearance of a character (and accentuation of another pre existing potentiality) the M must have evolved from N. The latter substance produces an antibody which can be absorbed completely by the substance M. M must, therefore, be contained in N. The character M, therefore, must have appeared after the disappearance of another character from N. Chronologically, then, N should come before M.

1931', V, Part 1, Calcutta, Central Publication Branch, p 529) and *common belief, the origin of this caste of* the physicians of Bengal is more mixed than that of the other high Hindu castes The author has found (unpublished work) such predominance of O, in this region of Asia, only in the Gurkha and the Burmese troops

(*iv*) The Hindu figures differ from those of Malone and Lahiri (R H Malone and M N Lahiri, 1929, *Ind Jour Med Res*, **16**, 963) for U P Hindus The difference lies in an increase in O This is contrary to the expectations based on Malone and Lahiri's conclusion to the effect that O increases as one travels up the Ganges valley The difference between the Brahmins at one end of the social scale and the depressed classes at the other is not significant The author, therefore, presumes that looking for minute differences, in small collections of figures, from odd places and professions in Bengal, is not likely to prove profitable

(*v*) The difference between the figures of the various Indian communities, Hindus and Mohammedans, with the exception of the Vaidyas and 'other castes' (a very small group) is not significant

The figures for Mohammedans in the author's collection are more representative of the general population than they would be when collected from small localities where inbreeding occurs This inbreeding, incidentally, is the essential difference between small and isolated Hindu and non Hindu populations Even when it occurs amongst the Hindus in Southern India and parts of the Bombay Presidency, it is much more restricted than it is amongst the non Hindus

(*vi*) The lack of significant differences between the various Indian communities in Calcutta further justifies the presumption that search for differences in small Bengalee communities Hindu or Mohammedan, is not likely to prove profitable

INTERNE, February 1949) has proposed the following classification

I Caucasoid group Frequency of rh highest (the peak reached in the Basques, *vide infra*) A_1 and A_2 present M a little more than N

II Negroid group Frequency of Rh_0 highest A_1 and A_2 present and also intermediate isogens M a little more than N

III Mongoloid group Rh_1Rh_2 highest, rh lowest A_2 absent

(1) Asiatic sub division M = N

(2) Pacific sub division (including Australian aborigines and Ainu) M low and N high

(3) American sub division (including Amerindians and Eskimos) M high and N low

These groups conform well with geographical groups of humanity and are likely to supersede all previous classifications The typescript prepared originally for this chapter has been reduced considerably for this reason

Mixing of genes carrying blood groups, etc — Rh + and Rh − being concerned in iso immunization, erythroblastosis and death of the fœtus are mutually antagonistic and therefore exclusive Every time an Rh − mother kills her baby through iso immunization, one gene for Rh and one for rh are lost If Rh be equal to rh no upset in the distribution of the type will occur If Rh be greater than rh then in course of time rh will be eliminated almost entirely Thus has rh been reduced, probably, in the Mongoloid group, indicating homogeneous mixture of the population occurring in many thousands of generations

Relatively high frequency of rh in the Caucasoid group indicates crossing of two or more original stocks,

Much support is lent to this argument by the fact that the predominant type in the Australian aborigines is N This stock is ancient and was isolated from their neighbours in Asia at an early period

If this argument be correct then what is now called N should be called M and what is now called M should be called N

Another problem is the composite nature of the present gene for N The compositeness is not so difficult to reconcile with the definition of a gene to day as it was before the recognition of Rh types

The antigen M is also a composite substance Fractions of it have been found in lower animals (*vide infra*)

Rh +/− State

The highest figure for the negative state is 15 per cent and obtains for the European stock, excepting the Basques (*vide infra*) The lowest is 1–2 per cent for the Chinese and the Negroes The Indian figure is 10 per cent This figure represents the absence of the standard antigen Rh_0 1–2 per cent of the subjects have in their blood antigen Rh′ and Rh″ only They are negative by the animal testing sera In terms of CDE, the D negative figure is 10 per cent

The Rh types have not yet been studied in India in a sufficiently large number The indication, however, is that Rh″ is a rare type Table IX, at the end of this chapter, gives Wiener's figures, based on the examination of bloods of 100 Indian sailors of the Indian Navy, in the U S A , during the World War II

Latest classification of humanity based on Rh, among other blood features —Wiener (A S , 1949, THE

Racial distribution of the P haemogen

Race	Number of subjects tested	Percentage	
		P +	P −
Caucasians	328	73.2	26.8
Negroes	73	97.7	2.3
Asiatic Indians	20	70.0	30.0
Mexican Indians	95	78.9	21.1

For other hæmogens recently described *see* pp 81 82 Their application in anthropology is not yet possible

Hæmogens in Lower Animals

O-A-B—Anthropoid apes are the only primates which have blood groups resembling O, A, B and AB

M—M of man, as has been stated before, is a composite antigen Old World monkeys (Ceropithecidæ) have most of the M antigens of man Lemurs have none at all Of the New world monkeys (Platyrrhini) the spider monkey only possesses some fraction of the antigen (Wiener, INTERNE, *loc cit*)

Rh—Human anti Rh sera do not agglutinate the rbc of *Macacus rhesus*, in spite of the Rh being derived from *rhesus* and the antiserum prepared against the rbc agglutinating the rbc of the majority of humans The situation does not appear to have been studied serologically yet

Hr—Chimpanzee rbc are agglutinated as strongly by the anti Hr sera as those of Rh negative humans The blood of this anthropoid species, therefore, contains a hæmogen closely allied to the one occurring in Rh − human subjects (Wiener, INTERNE, *loc cit*)

some with a high, others with a low frequency of rh The Basques for instance have the highest frequency of rh (53 7 per cent) and differ from other Europeans in their *mode of life and language significantly* They are an isolated people, inhabiting a triangular area in Spain, between France and Spain (The base of the triangle forms the Spanish side of the angle of the Bay of Biscay Each side is about 70 miles long and the total area is 2,739 square miles The population, according to 1931 census, was 891,710) The isolation is shown by their special costumes and language (Eskuara, Euskara or Uskara)

That B decreases from East to West in Eurasia is known Probably B was introduced into Europe by Mongolian invasions The isolated Basques were not affected appreciably by the introduction and have kept their frequency of B lowest in Europe (1 8) They are representative of the original Europeans

Rh incompatibility in marriage—Those who consider that this question falls under anthropology may go back to p 80

Hr +/− State

This constituent of the human rbc is included in the Rh state under Rh – Hr system or the CDE system

P +/− State

Neither the hæmogen nor the antiserum is freely available yet

The racial distribution, determined so far, for Caucasians, Negroes Asiatic Indians and Mexican Indians, is given in the following table compiled from Wiener's *published work (A S Wiener, E B Sonn and* R B Belkin, 1945, *Jour Immunol*, **50,** 341, A S Wiener, J P Zepeda, E B Sonn and H R Polivka, 1945, *Jour Exper Med*, **81,** 559)

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TABLE V

Blood groups of 2,472 subjects of Calcutta population

Communities	Total subjects	Groups								Genes			
		O		A		B		AB		p + q + r = 1.0			
		Total	Per cent	Total	Per cent	Total	Per cent	Total	Per cent	p	q	r	p + q + r
Europeans —													
Loyal Regt and K R R C	48	21	50.0	21	43.75	2	4.16	1	2.08	0.264	0.032	0.707	1.003
Others (mercantile community and domiciled Europeans May include Anglo Indian)	440	189	42.9	143	32.5	84	19.09	24	5.45	0.213	0.132	0.656	1.001
TOTAL EUROPEANS	488	213	43.6	164	33.6	86	17.6	25	5.12	0.218	0.121	0.660	0.999
Anglo Indians —													
I.M.D students	67	27	40.29	28	41.79	10	14.92	2	2.98	0.257	0.094	0.632	0.983
Others (European ancestry may be remoter)	279	102	36.5	103	30.9	57	20.4	17	6.09	0.245	0.143	0.604	0.992
TOTAL ANGLO-INDIANS	346	129	37.2	131	37.8	67	19.3	19	[illegible]	0.248	0.134	0.608	0.990

Indians —													
(i) Hindus Brahmin	201	76	37.8	40	19.9	71	35.3	14	6.9	0.145	0.240	0.615	1.000
Vaidyas	50	32	64.0	8	16.0	9	18.0	1	2.0	0.091	0.106	0.8	1.000
Kayasthas	149	53	35.5	29	19.4	60	40.2	7	4.6	0.128	0.258	0.59	0.982
Other high caste Bengalees (exact caste not determinable from records Includes Vaisyas in addition to the above three castes)	504	165	32.7	109	21.6	182	36.1	48	9.5	0.169	0.263	0.571	1.006
Depressed class Bengalees (scheduled and non-scheduled)	160	62	38.7	38	23.7	49	30.6	11	6.8	0.168	0.210	0.621	1.002
Non Bengalees	238	84	35.3	60	25.2	80	33.6	17	7.1	0.169	0.223	0.594	0.986
Total Hindus	1,302	469	36.02	284	21.8	451	34.6	98	7.5	0.160	0.238	0.6	0.998
(ii) Mohammedans	321	95	29.5	70	21.6	117	36.4	30	9.3	0.188	0.265	0.543	0.996
(iii) Other communities (Parsis Jews and Indian Christians)	15	7	46.6	5	33.3	2	13.3	1	6.66	0.225	0.106	0.683	1.014
Total Indians	1,638	571	35.03	368	22.5	570	34.9	129	7.9	0.163	0.242	0.592	0.997

Total Calcutta population tested 2,472 (Indians 1,638 Non Indians 834)

(S D S Greval and S N Chandra 1940 *Ind Jour Med Res*, **27**, 1112 1113 with the permission of the Editor)

TABLE VI

Figures for comparison from previous work

Population	Author	Number tested	Percentage of groups				Genes $p + q + r = 1.0$			
			O	A	B	AB	p	q	r	1.0
English London	Kirwan and Taylor	500	40.4	46.8	9.6	3.2	0.293	0.060	0.636	0.989
	Taylor and Ikin (1939)	1 073	45.9	42.0	9.3	2.7	0.257	0.062	0.677	0.996
(troops)	Hirszfeld and Hirszfeld	500	46.1	43.4	7.2	3.0	0.268	0.052	0.681	1.001
Australia	Tebbut	1 176	52.6	36.8	7.4	3.0	0.225	0.055	0.726	1.001
British troops Kasauli	Malone and Lahiri (1929)	147					0.317	0.032	0.652	1.001
Germans Berlin	Schiff	5 621	35.1	42.6	14.9	6.4	0.283	0.113	0.601	1.0
Munich	Kruse	1 300	42.5	43.0	9.0	1.9	0.269	0.067	0.653	0.989
Vienna	Corvin	6 934	34.4	45.2	15.0	5.4	0.301	0.112	0.587	1.0
Hindus various castes from U.P.	Malone and Lahiri (1929)	2 357	30.2	24.5	37.2	8.1	0.179	0.261	0.549	0.989

$p + q + r$ changed to unity from 100 of some records.

(S D S Greval and S N Chandra 1940 *Ind Jour Med Res* **27,** 1114 with the permission of the Editor)

TABLE VII

Groups and Types of 300 Indians in Calcutta

Groups	Types		
	M	N	MN
O 80 (26.7%)	33 (41.2%)	10 (12.5%)	37 (46.2%)
A 80 (26.7%)	31 (38.7%)	11 (13.7%)	38 (47.5%)
B 113 (37.7%)	51 (45.1%)	11 (9.7%)	51 (45.1%)
AB 27 (9.0%)	13 (48.1%)	0	14 (51.8%)
Totals 300	128 (42.7%)	32 (10.7%)	140 (46.7%)

(S D S Greval S N Chandra and I S F Woodhead 1939 *Ind Jour Med Res* **26**, 1048 with the permission of the Editor)

TABLE VIII

Types, M-N, for comparison

Author	Population	Number tested	Frequencies of types (percentage)		
			M	N	MN
Landsteiner and Levine*	New York —				
	Whites	532	26 1	20.3	53.6 (±1.50)
	Coloured	181	27 6	24.9	47.5
	American Indians	(i) 81	62 9	3 7	33.3
		(ii) 124	58 0	5 6	36.3
Schiff*	Germans	3,333	30 9	19 6	49 4
Crome*	Germans	1,300	32 5	18.5	49 0
Kerovitch*	French	400	33 0	21 2	45.8
Harley (loc cit)	English	200	32.5	19.5	48.5
Boyd and Boyd (W C Boyd and I G Boyd 1938 *Jour Immunol* **34**, 441)	Rwala Bedwin	208	57.5	5.8	36 7
	Calcutta —				
The Author (1939 *Ind Jour Med Res* **26** 1054)	Indians	300	42 7	10 7	46 7
	Europeans	50	30 0	18 0	52 0

* Quoted from A S Wiener 1935 BLOOD GROUPS AND BLOOD TRANSFUSION Bailliere Tindall & Cox London Only one figure after the decimal taken

TABLE IX

Rh Types of 100 Indians

Negative	Rh	Rh	Rh Rh	Rh	rh	rh
7 1	70.5	5 1	12.8	1 9	2 6	0

(From A S Wiener E B Sonn and R B Belkin 1945 *Jour Immunol* **50** 341)

APPENDIX I

THE BLOOD TRANSFUSION SERVICE AND BLOOD BANKS IN INDIA.

(From *The Indian Medical Gazette*, Vol LXXXII, No 8, August 1947, page 483)

For the purpose of supplying suitable donors of blood for special cases in hospitals and nursing homes a blood transfusion service was commenced in Calcutta 25 years ago Later, this service was enlarged Later still a blood bank was opened During World War II a serum bank for freeze drying serum was added at a special cost for the needs of the Armed Forces Similar institutions were established at Bombay, Madras, Lucknow, Patna, Lahore and Shillong For the needs of the civil population elsewhere in India the arrange ments obtaining in Calcutta prior to the serum bank will be described and the present day requirements added

The beginning —A service of supplying donors of blood, after grouping and matching directly the bloods of the prospective donors and recipients, was started by Lieut Colonel R B Lloyd, the then Imperial Serologist, in his laboratory in Calcutta in 1924 This was the first blood transfusion service in India It was meant for a small number of patients The donors were recruited from the European and Anglo Indian communities only and the fee was rather high for those days (Rs 50) A register of donors was maintained and a record of physical, serological and hæmatological examination before and after each service was kept

The enlargement —The author who followed Lieut Colonel Lloyd enlarged the service in 1935 by recruiting Indian donors as well The fee then could be reduced in deserving cases Further, donors of all blood groups could be selected more easily

Easy availability of blood steadily increased the demand for blood in Calcutta A still further enlargement was then effected in 1939 with a view to meeting this demand and making provision for national emergencies as well The usual register and records were maintained and it is worthy of record that during seven years, 1934–41, no donor suffered in health due to donation of blood This service supplied donors for 366 transfusions in 1940 The population served was of the order of two million

The blood bank—This service was started in 1939 by the author to supplement the service of donors of blood The total number of persons, in addition to the members of the Imperial Serologist's staff, who took part in the organization of the bank was 16 Out of them only 3 were whole time workers paid from a fund raised by the Bengal Red Cross Society Total salaries were of the order of Rs 1,700 a year Total cost of the equipment was Rs 1,100, recurring expenditure being only Rs 200 This was the first blood bank in India

A relation's or friend's blood found incompatible in grouping for one prospective recipient was taken in a bottle, kept in a refrigerator and given to another recipient of the same group The latter's relation's or friend's blood was also taken and, if found compatible, given to the first prospective recipient Whenever possible, for one blood supplied two bloods were taken as some bloods were discarded due to a positive or doubtful Wassermann reaction

Later, public spirited free donors were persuaded to call every six weeks or so to part with 400–500 cc of blood They were put on a register and could be sent for specially, if necessary Now an interval of three months is considered to be safer

New professional paid donors of the donors' service (who were sent to give blood on the spot in hospitals and

nursing homes) also gave a bottle of blood free to the bank This constituted a test of suitability as donors

[Some donors are known to collapse after giving varying quantities of blood For their benefit (i) ampoules of adrenaline chloride, 1 in 1,000, (ii) aromatic spirit of ammonia, and (iii) lemonade should be at hand]

Blood was also bought from prisons (for a nominal fee of Rs 5 plus rest for 2 days plus a remission in sentence)

Hæmatological observations were made on all bloods collected Two facts need special mention (1) Some donors' citrated blood did not show any visible change after several weeks' storage This was observed repeatedly (2) On the whole the specific gravity of the blood from the prisons was higher than that of the blood of the same community outside This could only be accounted for by the balance in diet, rigidly attended to in prisons

The blood was stored for not more than ten days in the beginning and for not more than seven days later Mostly it was used within 3 days It was stored by (i) communities and (ii), unlike the then London blood banks, by groups Universal donors' bloods of two qualities depending on the titre of the iso(hæmaggluti)nins and with a limit of 300 cc for the second quality were also available They were kept in distinctive containers and could be issued at a moment's notice

In the middle of 1941 about 200 donors of all classes could be bled for a national emergency every six weeks

The plasma bank—From blood remaining in storage longer than seven days plasma was separated and made safe for transfusion (*vide infra*)

The serum bank—This was essentially a war-time establishment and would be rather a wasteful way of providing material for transfusion in peace time Bloods were taken and allowed to clot Clear sera were pooled, filtered and freeze dried by special machinery

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The plasma, a by product in a blood bank, can be dried like serum also and waste avoided

The war time dried serum stocks are still available in most countries They are not likely to be replaced when expended

Rhesus grouping —The double blood transfusion service of pre war Calcutta (donors, and bottled blood and plasma) can be easily established in all big towns in India where laboratory facilities exist All the donors now, however, should also be rhesus grouped Most of the reactions due to the Rh incompatibility are due to the presence or absence of the antigen Rh_0 which can be detected by an animal serum Human testing sera so far have not been collected in India American sera (kindly supplied by Dr Wiener) have been used for special cases for several years in the laboratory of the Imperial Serologist (now Serologist), Government of India, Calcutta

Rh — subjects (by animal serum) should not be given whole blood at all, lest some donors negative by the animal serum should be positive by human serum (*i e* contain Rh′ and Rh″ antigen)

If human testing sera against Rh and Rh″ are available the correct type can, of course, be determined and a whole blood transfusion given

Even after using human sera, all Rh — subjects who have had a previous blood transfusion and all Rh — females who have borne children should have special attention paid to cross matching test the action of their sera on the rbc of the donor should be studied in a conglutination test after at least one hour's incubation A prospective recipient Rh — by animal serum should, of course never receive blood from a prospective donor Rh + by animal serum

Further, all ±, doubtful, reactions by animal serum should be looked upon as negative in a recipient and as positive in a donor

A subject ORh — (negative with human sera as well) with low isonin content has been considered safe all round Such, however, is not the case The donor may iso immunize an Hr negative subject The same observation applies to Rh — subjects of other groups for these groups An Rh — donor should only be used for an Rh — recipient unless, of course, the exact type can be determined and the Hr incompatibility ruled out

It has been suspected that there is a boost in blood transfusion (F K Albrecht, 1946, Modern Management in Clinical Medicine, Baillière, Tindall & Cox, London) The boost should be discouraged The plasma (subject to the restriction under the new danger of homologous serum jaundice) or serum (reconstituted from freeze dried material) suffices in most cases

Homologous serum jaundice —The new danger of homologous serum jaundice introduces a risk in plasma transfusion like the risk of Rh incompatibility in whole blood transfusion The risk really is greater inasmuch as it cannot be foretold All donors with a history of jaundice must, of course, be excluded

Probably protein hydrolysates will ultimately be found to be more suitable for intravenous alimentation than blood, serum or plasma

When the rbc must be used, it should be possible to remove all traces of plasma from them

For details of the jaundice *see* Appendix II

Points in procedure (1) Collection of blood — As first described by the author a Potain's aspirator available in most hospitals in India suffices for an apparatus The stopper carrying the essential parts fits an ordinary bottle quite well In the bottle are sterilized as many cc of a 3 per cent solution of sodium citrate as multiplied by 10 give the total quantity of blood taken in the bottle (20 cc for 200, 30 for 300 and so on) A loose knot on the rubber tube connecting the pump to

the bottle prevents the entry of contaminants from the pump which is not sterilized The rest of the apparatus is sterilized This method was re described later by other workers (L E Napier and C R Das Gupta, 1941, *Indian Med Gaz*, **76,** 742) Recently a 2 0 to 2 5 per cent solution of disodium hydrogen citrate has been preferred It does not char glucose if the latter is to be added for the preservation of the red blood cells (disodium hydrogen citrate 2 0 to 2 5 per cent 100 cc plus glucose 15 per cent 20 cc, total 120 cc, which is sufficient to prevent clotting in 400 to 420 cc of blood) and a slightly acid reaction of the mixture further aids the preservation (W d'A Maycock, 1947, Ministry of Health Bulletin No 14, *The Pharmaceutical Journal*, **158,** 22) See also Appendix III

(2) Preservation—The special stopper of the apparatus is replaced by an ordinary glass stopper with sterile precaution and the bottle after inscription (with Indian ink, covered with a layer of collodion) is left at room temperature to complete half an hour of this temperature The blood left in the tube is preserved in two lots (*i*) in sodium citrate solution for the red blood cells and (*ii*) in a dry tube for serum, for cross matching the recipient All the containers of blood are left in a refrigerator (one free from vibrations—electrolux—preferred) at about 4 to 8°C, the usual temperature outside the freezing chamber

Blood preserved for less than a week is as useful as fresh blood, for most purposes After a week its plasma should be collected and stored as a liquid up to 18 to 24 months or as a freeze dried powder for at least 5 years (Maycock, *loc cit*) Preservation of the red blood corpuscles for longer periods, with the aid of glucose, is really not necessary for banking blood, although the method is used for keeping blood for experimental purposes and for the manufacture of antisera against certain hæmagglutinogens Recently this method has

been described again (V R Khanolkar, 1946, *Indian Med Gaz*, **81**, 32)

Pooling of plasma from several donors of different groups makes it safe by lowering its isonin content On the other hand, the virus of homologous serum jaundice is thus spread over a larger number of recipients A mixture of two bottles, from subjects A and B, will yield a safe plasma as a rule

ADDITIONAL NOTES ON BLOOD, BLOOD PRODUCTS, PLASMA SUBSTITUTES AND BODY TISSUE KEPT IN A BLOOD BANK

(Based on BLOOD TRANSFUSION by E L De Gowin, R C Hardin and J B Alsever, 1949, *loc cit* and current literature, with a view to supplementing the author's scheme)

Whole blood 1 Fresh blood—The blood taken from the donor must be administered to the recipient *within a few hours* The American practice is to place it in the refrigerator (5°C to 10°C) at once during the interval (The author keeps the bottle for storage at room temperature for half an hour with a view to sub mitting the possible contaminants to the action of fresh blood He had by this technique no difficulty in maintain ing sterility Bottles of broth media handled as much as these bottles are not likely to remain sterile)

2 Preserved blood—It is blood taken in a solution containing an anticoagulant and dextrose, kept at 5°C to 10 C for *longer than a few hours* In it the platelets and leucocytes begin to disintegrate and usually disappear by the 5th day The rbc remain useful for 14 to 30 days Transmission of diseases by blood decreases with keeping

(ACD Acid Citrate-Dextrose, is a recently devised preserving solution Trisodium citrate 3 2 gm , citric

acid 0 36 gm, anhydrous dextrose 3 5 gm and water 131 cc, for 500 cc blood)

3 **Stored blood**—It is blood taken in sodium citrate only and kept at 5°C to 10°C for longer than a few hours Disintegrative changes in the rbc develop rapidly without dextrose It should not be used for whole blood after the 5th day It can be considered as good as preserved blood for 3 days only

Citrated plasma 1 **Liquid plasma**—With 5 per cent dextrose it stays good at room temperature for at least 3 years and is useful for treating shock and hypoproteinæmia Complement and prothrombin deteriorate rapidly after 3 days (after 10 days in a refrigerator) Antibodies are lost after 6 months Dextrose prevents precipitation of fibrin at room temperature (but not in a refrigerator)

Ultra violet irradiation of the pooled plasma, as a routine, for the inactivation of the virus of homologous serum jaundice has not yet been developed

2 **Frozen plasma**—If separated from rbc within 3 days and frozen forthwith, it does not lose any constituents The storage is done below − 20°C Frozen plasma may or may not contain dextrose

At this temperature the plasma is known to keep for 3 years at least Probably it keeps indefinitely If the temperature rises to − 15 C, the plasma will remain frozen of course but the labile components will disappear

The solid plasma is thawed at 37 C in a water bath and used at once

3 **Dried plasma.**—When freeze dried 3 days after the collection of blood, it has been found to be stable for at least 5 years It is converted into a liquid by adding the necessary quantity (stated on the container, equal to the original volume freeze dried) of 0 1 per cent solution of citric acid (to compensate for the loss of CO_2, which

also occurs during evaporation of water) The quantity may be reduced if a greater concentration of plasma protein is required (double strength has been advocated in treating œdema)

(If the blood was collected in a preservative solution containing sufficient citric acid, for example ACD solution, the addition of citric acid to the water is not necessary)

Use of complement—One possible reason for the failure of a crisis in some cases of pneumococcus pneumonia, in spite of the antibodies, was a deficiency of complement (G Taplin, 1940, *Journal of the American Medical Association*, **115**, 1676) This has been found to exist in several acute infections and in severe manifestations of allergy (L L De Gowin *et al*, 1949, *loc cit*) In fresh plasma and in frozen plasma (kept continuously below — 20°C) complement is available

Anterior Pituitary Hormone—Blood from pregnant women has recently been used in treating rheumatoid arthritis The action is obviously due to a hormone, presumably ACTH, which stimulates the production of compound F from the adrenals This compound is closely allied to compound E, cortisone, which cures all rheumatic states (*Indian Med Gaz*, 1950, **85**, 159) The selection of the donor will need care Bleeding may induce abortion, apart from affecting the expectant mother and the fœtus through loss of a small volume of their blood

Separated rbc—In collecting plasma rbc are left behind They can be used for transfusion up to 5 days after the collection of blood and even longer if the original blood contained dextrose Preserved with corn syrup (rbc from 500 cc of whole blood + 250 cc of 10 per cent corn syrup) they can be used for as long as 21 days The composition of the corn syrup used in America is 17 7 per cent dextrose, 16 8 per cent maltose, 16 2 per

cent higher sugars, 29 6 per cent dextrins and 19 7 per cent water

The rbc are transfused like the whole blood after all the necessary tests of compatibility It must be recalled that the original transfusion in World War I aimed at administering rbc only For diseases needing rbc only the whole blood is unnecessary

The rbc are also being used in dressing wounds as paste, powder or ointment (1) The paste It is made by pooling aseptically deposits from several lots of blood and storing them in a refrigerator for 10 to 20 days for disintegration of rbc It can be stored under refrigeration indefinitely Its application aids the growth of new tissue in open joints, infections, burns and chronic ulcers (including trophic, decubitus and diabetic ulcers) (2) The powder It is made by drying rbc aseptically either like dried plasma or like dried milk The former process yields a cake which is then powdered The powder is kept in sterile phials It is dusted over wounds (3) The ointment It is made from the powder For this purpose the following base has been recommended —

Cetyl alcohol	15 0 gm
White wax	1 0 gm
Propylene glycol	10 0 gm
Sodium laurel sulphate	2 0 gm
Water	72 0 cc

Substitutes for plasma 1 Serum albumin — It is a fraction of normal human plasma and is unstable in liquid form It is prepared as a 5 per cent or 25 per cent solution The stronger solution has the viscosity of blood

(Fractionation of plasma is carried out in a cold room at — 5 C, by the Cohn method, with the addition of alcohol in varying proportions The alcohol does not denature proteins below the freezing point of water)

2 **Gelatine solutions** —(1) Ordinary gelatine It is prepared specially for intravenous use by 'degrading', by autoclaving (Slightly degraded preparation—autoclaved for 20 minutes—is a solid gel at room temperature and must be heated before use, the preparation degraded further—autoclaved for 80 minutes—is liquid at room temperature) It is the best plasma substitute in 4 to 5 per cent solution Disadvantages are high viscosity at room temperature, rapid urinary excretion and interference with blood grouping and cross matching because of excessive rouleau formation If it is used in an emergency, the grouping should be done first and samples of blood kept for cross matching later, if necessary It is useful in shock but not in hypoproteinæmia (2) Fish gelatine, isinglass It has been used in the place of the other gelatine in concentration of 5 to 7 per cent

3 **Globin** —It is prepared from human hæmoglobin from discarded rbc The latest preparation (M M Strumia, 1946, *Am J M Sc*, **221**, 51) appears to be a very good substitute for plasma It is useful in the treatment of shock as well as of hypoproteinæmia, is cheap and easy to prepare, and is not toxic or antigenic

4 **Acacia** —It is deposited in the body and is now considered harmful

5 **Pectin** —It is also deposited in the body and cannot be recommended

6 **Methyl cellulose** —It has been used experimentally in dogs It is definitely toxic and cannot be recommended

7 **Collodin** —It has been used

8 **Bovine albumin** —It has not been found satisfactory

9 **Despeciated bovine serum** —It was tried during the war in England The despeciation was affected by

formalin and heat In samples prepared in India, according to the same method, the author found the reaction of the species with his antiserum

10 **Dextran** —This glucose polymer is not toxic or antigenic and has been used with success (*Lancet, i*, 22nd January, 1949, p 151)

The elimination of the substance occurs in the lower part of the intestine and is likely to affect adversely local conditions in disease (L Engstrand and B Aberg, 1950, *Lancet, ii* 1071)

Antihæmophilic globulin —This important plasma fraction reduces the bleeding time of hæmophiliacs for several hours The substance is precipitated with fibrinogen and then isolated by a process depending upon heat (Editorial, *J Amer Med Assoc*, 1947, **135,** 34)

Gamma globulin —This fraction of the globulin is present in plasma of normal subjects who have had the common infectious diseases and includes antibodies against diphtheria, scarlatina, virus A influenza, typhoid fever and measles It can be prepared from pooled plasma (C A Janeway, 1947, *New York State Journ Med*, **47,** 1357)

Fibrin film —It has been used in brain surgery It provides adequate closure and disappears, later, without producing reaction and adhesions

Fibrinogen and thrombin solutions —They are stable when dried from a frozen state Together they form a coagulum on a bleeding surface and stick tissue like skin graft (one of them is applied to bare area and the other to the graft)

Fibrin foam, made by mixing the two reagents, can be dried and sterilized It is soaked in a solution of thrombin and applied as required

Storage of other human tissues and products based on the hæmogen combinations —Other human tissues and products for storage in the blood bank are (1)

Human milk Such milk banks have been started in America When there is reason to believe that incompatibility of blood between mother and infant is responsible for the infant's inability to digest the mother's milk, milk from a compatible source should be available (2) Human cornea Eye banks have also been started in America Probably perfect compatibility of blood between the donor and the recipient would yield better results than those obtained so far (3) Human skin banks are the latest venture Skin has been sent by post Compatibility of blood should be an advantage (4) Human bone Bone banks have not yet been heard of Compatibility of blood should be an advantage (5) Human adrenal gland Successful transplantation has been reported A compatibility in blood would be an advantage (6) Human gonads A compatibility in blood would be an advantage The last five tissues can be obtained easily from healthy subjects who have died unnatural deaths (accidents, execution and deaths in battle)

Supply from a central blood bank—With suitable arrangements for cooling all materials can be supplied from a central bank over a considerable area Whole blood, however, will need special handling Agitation must be minimized

Price of blood—The average price per 100 cc, paid to the professional donor, in the U S A, in 1949 was 5 dollars The price for blood in Australia is nil Blood donation has become a social institution (Medical News, *Indian Med Gaz*, 1950, **85**, 465) The author believes that something similar, if not exactly identical, can be instituted in India also

Special glass for low temperature—The glass usually recommended for storage of blood and plasma suffices It is clear, colourless glass, Type I USP (De Gowin *et al*, *loc cit*) As a matter of fact, any good

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APPENDIX II

HOMOLOGOUS SERUM JAUNDICE

(From *The Indian Medical Gazette*, Vol LXXXII, No 4, April 1947, page 213)

The identity of this disease has been established during the last 10 years The term homologous applied to the serum indicates merely that it is derived from the same species There is no suggestion in it of its iso hæmagglutinin content *Isoserum* would be better than *homologous serum*

(The term homologous itself, applied to a *specific* serum in immunology, is not used in the correct biological sense The wing of a bat or even the wing of a bird is *homologous* to the arm of a man The clitoris is homologous to the glans penis The wing of a bird, on the other hand, is only *analogous* to the wing of a housefly Homology is an affinity between developmentally related structures which look dissimilar as opposed to analogy which is a resemblance in appearance and function only)

The term jaundice is rather euphemistic The morbid state arising from the parenteral entry of an isoserum is a serious form of hepatitis and much more likely to prove fatal than the 'catarrhal jaundice' of convenience

The available evidence suggests that the causative agent, probably a virus, is not the same as that causing the naturally occurring infective hepatitis (F O MacCallum, 1946, *Proc Roy Soc Med*, **39**, 655)

The jaundice transmitted by syringes, the late hepatitis following injection of any therapeutic agent, is identical with homologous serum jaundice and is communicated by traces of blood transferred on syringes

and well annealed glassware can be used as a container for liquids at $-20°C$ or even lower, provided it is sufficiently well insulated from the atmosphere so that it does not crack due to thermal shock caused by a marked difference in temperatures outside and inside the vessel

disease (MacCallum, *loc cit*) Very small quantities of serum (0 01 cc) can infect

The natural communicability of the disease, if it be naturally communicable at all, is of a very low order The incubation period is very long, of the order of months (Bradley, *loc cit*)

Similar disease in animals—Jaundice with mad staggers has been reported in horses after immunization with antigens administered in horse serum (Theiler, Gordon, March and Slagsvold, quoted by Bradley, *loc cit*)

Treatment—Two apparently hopeless cases of serum hepatitis have been treated successfully with protein hydrolysates intravenous 'alimentation' (500 cc, equivalent of 25 grammes protein, preceded by 500 cc of 5 to 10 per cent glucose) for several days (D J Simon and D Brown, 1946, *Lancet*, *i*, 492, H E Magee, 1946, *Proc Roy Soc Med*, **39**, 657) Large doses of methionine (2 5 to 3 grammes orally) have also been found useful (J Beattie and J Marshall, 1944*a*, *Nature*, **153**, 525, J Beattie and J Marshall, 1944*b*, *Brit Med J*, *ii*, 651, J Beattie, 1946, *Proc Roy Soc Med*, **39**, 658) This drug, however, has not been found useful in the impairment of liver function by other workers (C D Cook and F W Hoffbauer, 1946, *Journ Lab & Clin Med*, **31**, 56)

A new risk in blood transfusion—In blood transfusion to the usual risks—incompatibility of the red blood cells and sera, syphilis and malaria—must be added the new risk of the homologous serum hepatitis As a safeguard it has been naturally suggested that the donor should not have suffered from jaundice (H E Snyder, 1947, *J Amer Med Assoc*, **133**, 219) This precaution will exclude all previous sufferers from jaundice including those who might have suffered from the variety which on inoculation into other subjects might give rise to the homologous serum jaundice But it has not been

and needles from man to man (Memorandum by Medical Officers of the Ministry of Health, England, 1945, *Lancet*, ii, 116)

Cases in children attracted attention in the beginning —In 1937, 4 children died in or near Oxford, after receiving injections from a single batch of measles convalescent serum, nine or ten weeks previously Eventually 109 recipients of the serum were traced, 41 had been ill subsequently, 37 of them with jaundice Eight had died of hepatic necrosis All deaths had occurred between the 61st and 93rd day after receiving the injection (MacNatty, quoted by W H Bradley, 1946, *Proc Roy Soc Med*, **39**, 649)

Cases in adults attracted attention later —The American troops receiving **yellow fever vaccine** developed jaundice This was not due to the yellow fever virus but to the human serum used as a vehicle of the virus (Findlay, 1940, quoted by Bradley, *loc cit*) The icterogenicity was confined to certain batches of the vaccine with adjacent serial numbers

Mumps serum jaundice attracted attention in England In a unit of a British Tank Corps two batches of pooled serum, from 11 convalescent cases, were given to 260 cases once and to 204 of the same men twice at 14 days' interval Two hundred and twenty men were followed up, 44 7 per cent developed jaundice 44 to 123 days later (P B Beeson, G Chesney and A M McFarlan, 1944, *Lancet*, i, 814)

Pappataci vaccine jaundice was noticed in Southern Russia The active virus had been mixed with convalescent human serum

Jaundice after **blood, plasma** or **serum** transfusion has been noticed in England in many cases, 2 to 4 months after the transfusion Deaths have occurred from hepatitis No previous treatment of the whole blood plasma or serum appears to be effective in preventing the

strangers The history of the former is better known, and because of a similar environment, the recipient and the donor are likely to have a similar immunological constitution

No Indian cases—Post transfusion homologous serum jaundice has not attracted attention in India yet The Calcutta Blood Bank provided blood and serum for 6,762 transfusions during 1945, 1946, and a part of 1947, mostly to hospitals, and did not receive a single report of a recipient developing jaundice after a month or so (D N Chatterji, 1947 personal communication) It is quite possible that the disease, like many other European diseases, does not thrive on Indian soil (S D S Greval, 1946, *Indian Med Gaz*, **81**, 254)

A re estimation of the utility of blood and blood production—The use of convalescent serum for children cannot be considered safe any longer in Europe at any rate Measles could not have killed as many children at Oxford as were killed by the serum

The utility of transfusion, in civil practice, also needs a reconsideration It should be differentiated from a mere boost Such a boost in the transfusion of whole blood has been suspected and discouraged (F K Albrecht, 1946, Modern Management in Clinical Medicine Bailliere, Tindall & Cox, London, p 1156) It should be discouraged in the transfusions of other constituents of blood also

ADDITIONAL NOTES

Ultra-violet irradiation of plasma to control homologous serum jaundice—That ultra violet irradiation kills the virus has been established (A M Wolf *et al*, 1947, *J Amer Med Assoc*, **135**, 476) A practical method for employing the irradiation routinely, however, has not yet been evolved

Gamma globulin in treatment of homologous serum jaundice—It is of no value in treatment and of questionable value in prophylaxis

established that the donors of the sera which did give rise to the disease had suffered from jaundice

Substitutes for transfusion—Perhaps the protein hydrolysates could replace plasma and serum for most cases needing a transfusion The compatible red blood cells washed free of the serum in saline could be used for the whole blood, if the virus is found in the serum only As a matter of fact the preserved blood used in the World War I consisted of preserved red blood cells only (A S Wiener, 1943, Blood Groups and Transfusion Charles C Thomas, Springfield, Illinois, Baltimore, Maryland, p 135)

The serum can be eliminated by dilution with a large quantity of saline of a small quantity of whole blood The settled cells can be separated, re suspended in another large volume of saline and allowed to settle a second time If the supernatant fluid after the second settlement does not react with a strong antihuman serum, the chances of any serum being left after a third settle ment are negligible The average antihuman serum used for medicolegal purposes in India detects the human serum in a dilution of 1 in 40,000 (S D S Greval and A B Roy Chowdhury, 1947, *Indian Med Gaz*, **82**, 125) A stronger antiserum can be prepared for the purpose *A possible diffusion of the contents of the red cells into* the saline will not interfere by giving a false positive reaction This material does not react with the anti serum prepared against the serum (S D S Greval and A B Roy Chowdhury, 1945 *Indian Med Gaz*, **79**, 81)

Only human serum albumin may be used This derivative has been reported to be free from the icterogenic agent (D R Climenko, 1947, *New York State Journ Med*, **47**, 266)

Special selection of donors—When a real whole blood transfusion is considered absolutely necessary, members of the same family community and locality (in this order) are likely to provide safer donors than total

APPENDIX III

THE NEEDLE IN THE VEIN

(From *The Indian Medical Gazette*, Vol LXXV, No 2, February 1940, page 103)

The intention is to discuss ways of entering as well as of avoiding a vein, and also to comment on the sterility of the implement used

1 Should the vein be entered by a primary thrust, from above, through the skin and the wall of the vein together, or by a secondary thrust, from above or from one side, after the skin has been traversed ?

The trauma is minimum when the primary thrust is made from above, through the skin and the wall of the vein together This procedure is possible when the vein is fixed either naturally or by the finger of the operator

If the needle has traversed the skin and then travelled between the skin and the vein or to one side of the vein, it can be made to enter the vein by a secondary thrust from above or from one side The secondary thrust being more oblique than the primary one has a more tearing action The trauma is greater The procedure of choice, therefore, is the entry with a primary thrust from above

2 Should the oblique opening in the needle be looking upwards or downwards ?

The needle has a cutting edge (not a boring point) and takes a flap from the wall of the vein into the lumen In figure 11 the needle is introduced into the vein of the

ENTRY INTO A VEIN

WRONG

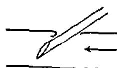


Fig 11

RIGHT

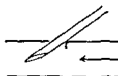


Fig 12

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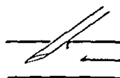


Fig 12

Record syringe needle which is usually used in giving intravenous injections (S D S Grewal and S N Chandra, 1940, *Indian Med Gaz* **75**, 11) Since then other workers' opinion to the same effect has become available (C R Boland, N S Craig and A L Jacobs, 1939, *Brit Med J*, *ii*, p 826, publication delayed in transit)

4 Special sites for puncture when the needle has failed to enter a vein at the usual sites

Attention is drawn to three special sites (*i*) for taking blood or giving an injection veins over the malleoli are available regardless of the obesity of the subject, (*ii*) in small children the external jugular vein can be entered, and (*iii*) for giving saline or blood, corpora cavernosa of the penis (L H Snyder, 1929, BLOOD GROUPING IN RELATION TO CLINICAL AND LEGAL MEDICINE, Williams and Wilkins Co Baltimore) can always be entered

A puncture in the fontanelle in infants is to be avoided

5 How unintentional entry into a vein can be avoided

(1) When giving an intramuscular injection, the needle should first be introduced and a dry puncture assured The syringe containing the medicament is then attached to the needle When the medicament is contained in a rubber capped bottle, yet another needle suitably stuffed with sterile cotton wool is necessary to admit air (filtered) into the bottle, to replace the volume drawn into the syringe

(2) When giving a subcutaneous injection needing speed, as is done in antirabies clinics, and involving regions which cannot be guaranteed to be free from venules, such as the abdominal region, it is better not to use a fine needle The chances of a needle with a large bore such as is used with a 20 cc Record syringe

arm, pointing towards the heart as is done in giving an intravenous injection, with the opening looking upwards The flap is bent with its *convexity* against the flow of blood (indicated by the arrow) On withdrawal of the needle such a flap may not fall back into position easily In figure 12 the needle is introduced in the same direction but with its opening looking downwards The flap is bent with its *concavity* against the flow of blood On withdrawal of the needle such a flap will fall back into position easily If the needle happens to puncture a valve in the vein, the consideration for the flap acquires added force

In giving an injection the introduction of the needle pointing towards the heart, apart from directing the injected fluid centrally, enables the operator to face the subject and watch the latter's face In taking blood from a vein it might be thought that the needle should be introduced pointing away from the heart and that the oblique opening should look upwards There is, however, no need to introduce the needle pointing away from the heart and lose the benefit of watching the subject's face With appropriate compression on the limb (not abolishing the pulse) the blood in the vein, being under pressure, flows regardless of the direction of the needle

It has been recommended (A S Wiener, 1935, BLOOD GROUPS AND BLOOD TRANSFUSION, Bailliere, Tindall & Cox, London) that in dealing with small veins the needle may be introduced pointing away from the heart It does not appear to be necessary to do this The procedure of choice, therefore, is the introduction of the needle pointing towards the heart with the oblique opening looking downwards

3 The bore of the needle

The writer in a recent communication suggested that the bore of the needle for taking blood from a vein for transfusion should not be larger than that of a 20 cc

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needle, of entering a venule are much less than those of a fine needle

(3) When giving a subcutaneous injection in the arm, pinching the skin and inserting the needle in the base of the fold are to be preferred to stretching the skin and thrusting the needle into it In the tissue over which the skin is stretched venules are flattened and are likely to be entered by a fine needle which is usually used In the tissue which is pinched the venules are stretched and narrowed They are more likely to be cut across than entered by even a fine needle Even for this injection the writer does not use a fine needle

6 Sterility of the needle

Sterility of the syringe was discussed in no less than nine communications, in the *British Medical Journal*, between 30th April, 1938 and 11th June, 1938—(*i*) Editorial Annotation (1938, *Brit Med J*, *i*, p 955), (*ii*) J S Crabbe (1938, *Brit Med J*, *i* p 1070), (*iii*) G R Murray (1938, *Brit Med J*, *i*, p 1178), (*iv*) J Jones (1938, *Brit Med J*, *i*, p 1178), (*v*) J Smalley (1938, *Brit Med J*, *i*, p 1179), (*vi*) Editorial Comment (1938, *Brit Med J*, *i*, p 1178), (*vii*) G B D Gray (1938, *Brit Med J*, *i*, p 1237), (*viii*) R A Manclark (1938, *Brit Med J*, *i*, p 1237) and (*ix*) N Pines (1938, *Brit Med J*, *i*, p 1292) It came up again as an integral part of a review of recent advances in vaccine therapy, in 1939 (A Fleming, 1939, *Brit Med J*, *ii*, p 99) The present writer adds the observation that a syringe and needle, howsoever sterilized, cease to be sterile when dried by sucking and expelling the ordinary air of the room He generally uses a Record syringe and his method of sterilizing is to (*i*) clean the syringe and the needle immediately after use, (*ii*) leave them for 15 minutes in 7 per cent phenol in water, (*iii*) transfer them to a pan of boiled water, (*iv*) dehydrate them by sucking and expelling absolute alcohol—second quality, kept for the purpose, (*v*) keep them assembled, ready for use, in

absolute alcohol in a metal case, and (ii) wash them by sucking and expelling sterile saline Once a month the metal containers are emptied into a jar to yield the absolute alcohol of the second quality and refilled The absolute alcohol of the second quality is also renewed once a month When a Roux's syringe is used, it is sterilized every day in hot oil (temperature 120° to 140°C) completely to begin with, i e all the parts excepting the top of the rod of the piston are dipped separately and then assembled Later, filling the syringe and the needle twice with the oil suffices Excess of oil is removed (if desired) by sucking air through a flame (for this step a guard covers the needle) and expelling it, or preferably by sucking and expelling boiling water

7 Prepuncture and postpuncture care of the skin, the vein and the subject

Painting liberally with a simple solution of iodine (B P) is preferable to swabbing with spirit or a solution of phenol The stain is removed (if desired) by swabbing with absolute alcohol Immediately before the withdrawal of the needle compression on the limb is removed and a swab (on a stick) soaked in the iodine solution is placed in contact with the needle to press on the puncture the moment the needle is withdrawn Bleeding, if any, is checked by pressure Flexible collodion (B P) is dropped on the puncture with a dropper to cover an area of the size of the thumb nail After a false skin has formed (which may be delayed in hot and wet months for over 15 minutes) the subject is allowed to depart with advice that the punctured limb should not be used in exertion of any kind for 24 hours

Prepuncture care is specially important when the subject is a donor of blood for cold storage The fact that accidents never (or hardly ever) occur with the therapeutic use of the needle, after a certain precaution has been taken, does not guarantee the bacteriological

sterility necessary in storing blood Stray micro organisms injected into the blood stream of living subjects are as a rule promptly destroyed, injected subcutaneously they are also destroyed with some effort on the part of the tissues, swept into blood taken for storage they may or may not be overwhelmed, falling into culture media they will grow as contaminants without fail, of course Liberties taken with the needle in therapeutical procedures will more often than not contaminate nutrient broth and agar The same remarks apply to the syringe

Incidentally, the syringe and needle which are filled with ordinary air in order to replace the contents of a rubber capped bottle are not, strictly speaking, sterile although they are more so than when they have been dried by repeated filling with air Sterility of the contents of a rubber capped bottle into which have been blown many syringes full of unfiltered air is always doubtful Needles with cotton wool in their upper ends are sterilized and kept for admitting air into such bottles

The swabs on sticks are sterilized and kept in separate test tubes

The subject, if a donor for cold storage, should attend after a very light meal, if not after only liquid refreshment (Chyle in the blood interferes with the examination of the contents of the stored bottle) He should be accommodated in a long chair in which he can be placed in a supine position, if necessary Aromatic spirits of ammonia (B P) and drinking water should be available Means of applying pressure over a hæmatoma should also be available Provision must also be made for dealing with hæmophiliacs

8 Importance of minor details

The large number of recent references embodied in this short communication is indicative of the importance of the items concerning which minor details have been given More veins have been pricked in England and

India during 1939 than ever before The practice is likely to last It is hoped that these remarks on minor details will add to the ease, safety and utility of the process of pricking veins and tissues

Summary

1 A vein is best entered by a primary thrust from above, through the skin and wall of the vein together

2 The needle should be directed towards the heart with its oblique opening looking downwards

3 A needle of a large bore should not be used for taking blood

4 When for any reason a needle cannot enter a vein at the usual sites, other useful sites for injection are (i) veins over the malleoli, (ii) external jugular vein—in small children, and (iii) corpora cavernosa of the penis The first two sites serve for taking blood also

5 Entry into a vein has to be avoided in giving injections subcutaneously and intramuscularly For the former the needle should not be extremely fine and the skin should not be stretched, but pinched For the latter the needle must be introduced alone and a dry puncture assured

6 A long list of recent references on sterility of the syringe (and of necessity of the attached needle) is available An additional observation is that the syringe and needle dried by sucking and expelling air from a room are not sterile

7 Prepuncture and postpuncture care of the skin and the vein are best assured by iodine before, and collodion and rest after the puncture Drinking water and aromatic spirits of ammonia should be at hand, and so should be the means of stopping bleeding from the puncture

8 Minor details in pricking veins and tissues have become more important recently

APPENDIX IV

THE cc, c cm, MIL, ML AND ml

(From *The Indian Medical Gazette*, Vol LXXXIII, No 6, June 1948, page 281)

Historical—The urge to adopt a natural length as a fundamental unit of measurement was felt as early as the 17th century The astronomer Jean Picard (1620–1682) proposed to take as a unit the length of a pendulum beating 1 second at sea level at a latitude of 45° Nearly a century later the National Assembly of France, in 1790, appointed a committee to consider the relative merits of (*i*) the pendulum, (*ii*) a fraction of the length of the equator, and (*iii*) a fraction of the terrestrial meridian The last measure was favoured and a commission appointed to measure the meridian between Dunkirk and Montjuich near Barcelona

The 'end standard' metre (metre des archives) — In 1799 a commission of the representatives of different countries, convened by the French Government, chose the length of the arc of the polar quadrant which passed through Paris as a natural length 1/10,000,000 of it they called the METRE This was a natural, invariable, measure A bar of platinum measuring exactly a metre was made and called the '*metre aes archives*' It was an 'end standard' representing the length from *end to end*

As a matter of fact the length of the arc was not measured absolutely accurately The length of the bar is less than what it was intended to be by about 1/8,000th of itself The bar, however, was not altered

The metre was divided decimally into decimetres, centimetres and millimetres

The measure of volume was a cubic decimetre and called a LITRE One litre = 1 decimetre × 1 decimetre × 1 decimetre = 1 cubic decimetre = 1,000 cubic centimetres = 1,000 cc 's = 1,000 cc ('s may be dropped)

The measure of mass was also derived from a cubic decimetre It was the mass (weight for all practical purposes) of a litre of distilled water of maximum density (*i e* at 4 C) and called a KILOGRAMME A cylindrical piece of platinum of this mass (the weight) was made, kept as a standard and called '*kilogramme des archives*' It was divided decimally into decagrammes, grammes, decigrammes, centigrammes and milligrammes

The 'line standard' metre (prototype metre) —In 1875 there came into being the *Convention des metre* and later *Bureau International des Poids et Mesures* It was felt that the original 'end standard' should be replaced by a 'line standard', because the latter could now be made more accurately than the former On a bar of iridium platinum (10 per cent Ir and 90 per cent Pt) two lines were ruled to mark as nearly as possible the length of the *metre des archives* The new measure was called the PROTOTYPE METRE

The prototype kilo —The *kilogramme des archives* was also changed The new weight, called the PROTOTYPE KILOGRAMME, was a cylinder of the same iridium platinum alloy as was used in the bar of the prototype metre and had a mass as nearly as possible of the *kilogramme des archives* It was no longer derived from the litre

The litre redefined —In 1902 the litre was redefined as the volume of the mass of one kilogramme distilled water at 4°C Thus, instead of a kilogramme being derived from a litre, a litre was derived from a kilo gramme 1/1,000 litre is now a MILLILITRE (MIL, Ml or ml), not a cubic centimetre (cc)

In changing things round in this way the post 1902 litre has been found to be slightly larger than the pre 1902 litre The ml is therefore slightly larger than a cc

1 ml = 1 00028 cc according to the British Pharmacopœia

On this obviously insignificant difference too there is a difference of opinion According to some workers,

1 ml = 1 00027 cc (J M Hamill, 1947, *Brit Med Journ*, 5th April, p 460)

Perhaps it is not quite so 'sad to reflect that, although nearly half a century has passed since the litre was redefined, many people, including regrettably a significant proportion of the medical profession, continue incorrectly to use the cubic centimetre as an alternative to millilitre' (Hamill, *loc cit*)

According to a medical lexicographer from the other side of the Atlantic

'mil' = thousandth part of a litre, a cubic centimetre (T L Stedman, Stedman's Medical Dictionary, 1933)

According to a recent encyclopaedic book on medicine The metric system is now official for the United States Pharmacopœia and publications of the American Medical Association (H T Hyman, 1947, An Integrated Practice of Medicine, W B Saunders Co, Philadelphia and London)

In the metric apothecary equivalents, however, cc, not ml is the equivalent of millilitre as given by the author

Nearly all syringes and most pipettes are marked in cc's

'c cm' is only a longer way of writing cc We prefer cc 'c c' (with a full stop after the first c) is not necessary Further, it appears to be an abbreviation for 'chief complaint'

MIL, mil, ML and ml denote a millilitre We prefer ml, if cc is not used

Metric system, and the gramme, cc and ml —It has been stated that the weights and measures can no longer be correctly described as metric inasmuch as the kilo and the litre have been legalized as weight and volume of a cylinder specially preserved (Hamill, *loc cit*) The prototype kilo, however, being 'as nearly as possible' equal to the *kilogramme des archives*, must be as nearly *metric as it is possible to be* It is, therefore, metric and will remain so The rest follows The ml must be metric and the cc of course is metric, being derived from the metre itself

Metric equivalents —Eminent British men of science have recently stressed the need of 'giving the metric equivalent of data expressed in British units' (R Robinson, H H Dale, E V Appleton, C G Darwin and C C Paterson, *Brit Med Journ*, 16th August, p 269, 1947) The position is particularly difficult with respect to ml and/or cc as the equivalents from the following sources show —

British Pharmacopœia Stedman (*loc cit*)	1 cc	= 16 68911 minims
Imperial Apothecaries measure	1 cc	= 16 23412 minims
US Apothecaries measure	1 cc	= 16 minims
Dorland W A N the American Illustrated Medical Dictionary (1947)	1 cc	= 16 minims
	1 fluid ounce	= 30 cc
Hyman (*loc cit*)	1 cc	= 16 minims
Tuberculin syringes	1 cc	= 16 minims

APPENDIX V

THE PRECIPITIN RING TEST FOR DETERMINING THE SOURCE OF BLOOD IN A STAIN

An extract in normal saline of the stain, of the order of a 1 in 1,000 dilution of the original serum in the stain, is superimposed on small quantities of known antisera (undiluted) A ring of white precipitate develops at the junction of the two liquids, with the appropriate antiserum

Preparation of anti human serum —Fresh pooled serum from at least 6 healthy donors of blood is filtered through Seitz filter and inactivated at 56°C for half an hour 3 cc are given intravenously to fowls (which have been observed for a week to exclude disease) The serum protein is the antigen

Ten days later, the fowls, after being kept on water (and watery vegetables like onions, otherwise the serum will be chylous) for 24 hours, are anæsthetized with ether and bled to death Each fowl yields about 20 cc blood which yields about 7 cc of serum The serum is separated, tested for **stability, sensitiveness** and **specificity** and stored frozen, if found suitable

A stable serum does not become opalescent in a 1 in 5 dilution in normal saline

A sensitive serum reacts with (1) a 1 in 1,000 dilution of the human serum, within 2 minutes frankly, +, and within 10 minutes sharply, + +, (2) a 1 in 20,000 dilution, in 10 minutes dubiously, ±, and in 20 minutes frankly, +, and (3) a 1 in 40,000 dilution, in 20 minutes dubiously, ±, otherwise the serum is either not sensitive or too

MIL, mil, ML and ml denote a millilitre We prefer ml, if cc is not used

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Tuberculin syringes	1 cc	= 16 minims

Other antisera against animals are prepared, tested and used in the same way

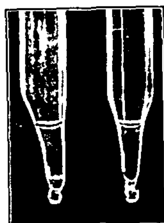


Fig 13 The Precipitin Reaction The left hand tube shows a Positive Reaction The right hand tube shows a Negative Reaction Photograph (From Lyon's Medical Jurisprudence for India 10th edition revised by the author in press)

As has been stated before, in determining the blood group it must first be established that

(1) The blood is present Chemical and spectroscopical tests must be applied

(2) The blood is human The test with anti human serum will decide It should be positive

(3) There is no admixture with animal blood Tests with anti sheep and anti fowl sera will decide They should be negative

For further details, see LYON'S MEDICAL JURISPRUDENCE FOR INDIA, edited and revised by the author, or SEROLOGICAL TECHNIQUE AND IMMUNOTHERAPY (in press), by the author

sensitive (excessive sensitiveness is reduced by aging in the cold—unfrozen)

A stable and sensitive serum is specific if it does not react with (1) buffalo's serum 1 in 1,000, (2) dog's serum 1 in 1,000, (3) horse's serum 1 in 1,000, (4) fowl's serum 1 in 1,000, (5) sheep's serum 1 in 1,000 and (6) an extract from dried stain of monkey's blood made to correspond to a 1 in 1,000 dilution of human serum by comparison of froth

[A 1 in 1,000 dilution of pooled healthy human serum in a standard test tube, when frothed by hitting against the palm of the hand, produces certain amount of froth An unknown dilution of sera can be matched against the dilution by the **froth index** in this **foam test** The test is used in preparing standard 1 in 1,000 dilutions of serum proteins from stains Of a good stain on cloth an area about 1 cm in diameter is cut out and extracted with 2 cc of normal saline in a test tube at room temperature for half an hour, without shaking (otherwise opalescence will occur and will render the extract unfit for the test) Less than half of the extract is transferred gently to another tube, diluted with 1 to 3 cc of normal saline and frothed More saline or original extract is added to obtain the required froth *The froth may be slightly thinner but not thicker than that of the standard*]

The test—In Fig 13 on p 197 is shown the ring in a tapering tube The extract for test is put in the tube and the latter made almost horizontal quickly for a fraction of a second to wet a surface above the level of the extract On this surface, with the tube at an angle of 45 degrees, is deposited a small quantity of the antiserum from a capillary pipette and the tube made vertical The serum sinks to the bottom The extract is thus superimposed Controls of the unstained material and of the extract with normal fowl serum are set up for comparison

10	GREVAL S D S	MAKING PLASMA SAFE FOR TRANSFUSION	*Indian Med Gaz* **75** 737 Dec 1940
11	GREVAL S D S CHANDRA S N AND WOODHEAD I S F	ON ISO HÆMAGGLUTINATION NOMENCLATURE TITRATION OF ISO HÆMAGGLUTININS NEED FOR REVISION OF TECHNIQUE OF GROUPING BLOOD ETC	*Ind Jour Med Res* **29**, 231 Jan 1941
12	GREVAL S D S	OBTAINING COMPATIBLE BLOOD BY EXCHANGE AND ASSOCIATED CONSIDERATIONS	*Cal Med Jour* **38**, 79 Feb 1941
13	GREVAL S D S AND CHANDRA S N	AN IMPORTANT ANTIGENIC DIFFERENCE BETWEEN HÆMAGGLUTINOGENS M & N	*Ind Jour Med Res* **29**, 517 1941
14	GREVAL S D S CHANDRA S N AND CHATTERJI D N	TAKING BLOOD FOR TRANSFUSION FURTHER IMPROVISATIONS	*Indian Med Gaz* **76**, 413 1941
15	GREVAL S D S AND CHANDRA S N	A NOTE ON BLOOD TRANSFUSION SERVICES OF CALCUTTA (ORGANIZATION ROUTINE AND RESEARCH)	Govt of Bengal publication 1941
16	GREVAL S D S	BLOOD BANKS	*Current Science* No 11 423 Nov 1942
17	GREVAL S D S	THE NEW Rh CONSTITUENT OF HUMAN BLOOD	*Ibid* **12**, No 8 222 Aug 1943
18	GREVAL S D S BHATTACHARJI J N AND ROY CHOWDHURY A B	ON ISO HÆMOLYSIS REPORTS ON TWO ISOLYSINS AND ASSOCIATED CONSIDERATIONS	*Ind Jour Med Res* **31**, 197 Oct 1943
19	GREVAL S D S BHATTACHARJI J N AND DAS B C	DETERMINATION OF BLOOD GROUPS FROM STAINS	*Ibid* **31**, 203 Oct 1943
20	GREVAL S D S AND ROY CHOWDHURY A B	THE Rh CONSTITUENT OF THE HUMAN RED BLOOD CELL	*Jour I M A* **13**, 65 Dec 1943

APPENDIX VI

AUTHOR'S PUBLISHED WORK ON WHICH THE BOOK IS BASED

The reader may consult the following papers for additional details on some items. References to other workers on the items will be found in the papers.

1	GREVAL, S. D. S., CHANDRA, S. N., AND WOODHEAD, L. S. F.	ON M & N IN BLOOD GROUPS: TECHNIQUE OF TYPING, ANTI-FLUIDS, FINDINGS IN 300 INDIANS AND ASSOCIATED CONSIDERATIONS	*Ind. Jour. Med. Res.* **26**, 1041, April 1939
2	GREVAL, S. D. S.	USE OF BLOOD TESTS IN EXCLUDING PATERNITY AND MATERNITY	*Indian Med. Gaz.* **74**, 388, July 1939
3	GREVAL, S. D. S., AND CHANDRA, S. N.	DIFFICULTIES AND DANGERS IN PROVIDING DONORS OF BLOOD	*Ibid.* **74**, 461, Aug. 1939
4	GREVAL, S. D. S.	THE NEEDLE IN THE VEIN	*Ibid.* **75**, 103, Feb. 1940
5	GREVAL, S. D. S., AND CHANDRA, S. N.	TAKING BLOOD FOR TRANSFUSION	*Ibid.* **75**, 11, Jan. 1940
6	GREVAL, S. D. S., AND CHANDRA, S. N.	BLOOD GROUPS OF COMMUNITIES IN CALCUTTA	*Ind. Jour. Med. Res.* **27**, 1109, April 1940
7	GREVAL, S. D. S.	THE USE OF BLOOD TESTS IN EXCLUDING PATERNITY AND MATERNITY (WITH A NOTE FOR NON-MEDICAL READERS)	*The Burma Police Jour.* **3**, 2, July 1940
8	GREVAL, S. D. S., CHANDRA, S. N., AND ROY CHOWDHURY, A. B.	TAKING BLOOD FOR TRANSFUSION (IN POTAIN'S ASPIRATOR): FURTHER DETAILS INCLUDING COLD STORAGE	*Indian Med. Gaz.* **75**, 513, Sept. 1940
9	GREVAL, S. D. S.	BLOOD GROUPS AND TYPES	*Current Science* **9**, 487, Nov. 1940

30	GREVAL S D S AND ROY CHOWDHURY A B	SEROLOGICAL TECHNIQUE CONSTITUTION OF BLOOD HEMAGGLUTINATION AND DETERMINATION OF GROUP OF BLOOD	*Ibid* **81**, 352 Sept 1946
31	GREVAL S D S AND ROY CHOWDHURY A B	SEROLOGICAL TECHNIQUE DETERMINATION OF THE TYPE OF BLOOD (M N SYSTEM) DETERMINATION OF THE Rh +/− STATE OF BLOOD DETERMINATION HOMOZYGOUS/HETEROZYGOUS STATE DETERMINATION OF OTHER CAUSES OF ERYTHROBLASTOSIS FOETALIS	*Ibid* **81**, 410 Oct 1946
32	GREVAL S D S AND ROY CHOWDHURY A B	SEROLOGICAL TECHNIQUE DETERMINATION OF BLOOD GROUPS FROM STAINS	*Ibid* **81**, 475 Nov 1946
33	GREVAL S D S ROY CHOWDHURY A B AND BANERJI B	A PRESUMPTIVE CASE OF ERYTHROBLASTOSIS FOETALIS DUE TO Rh INCOMPATIBILITY	*Ibid* **82**, 76 1947
34	GREVAL S D S AND ROY CHOWDHURY A B	SEROLOGICAL TECHNIQUE ADDENDUM TO Rh +/− STATE CONGLUTINATION IN DETECTING ANTI Rh BODY	*Ibid* **82**, 125 March 1947
35	GREVAL S D S ROY CHOWDHURY A B AND MUKHERJI N G	THREE CASES OF ISO IMMUNIZATION AGAINST Rh ANTIGEN FOUND IN FIVE WEEKS IN CALCUTTA AND ASSOCIATED CONSIDERATIONS	*Ibid* **83**, 370 1948
36	GREVAL S D S	SEROLOGICAL TECHNIQUE TECHNIQUE OF TESTING BLOOD (FOR Rh) WITH HUMAN TESTING SERA	*Ibid* **84**, 191 1949
37	GREVAL S D S AND MUKHERJI N G	LOW ISO(HEMAGGLUTINO)GEN CONTENT OF SALIVA IN CALCUTTA	*Ind Jour Med Res* **38** 1, Jan 1950

21	CRLVAI S D S BHATTACHARJI J N AND ROY CHOW DHURY A B	THE ISOLYSINS	*Indian Med Ga.* **78** 593 Dec 1943
22	GRFVAI S D S AND BHATTACHARJI J N	SOMI PHYSICAL AND IMMUNOLOGICAL DIFFERFNCES BETWEEN NATURAL AND RECONSTITUTED HUMAN SERUM	*Ind Jour Med Res* **32** 23 May 1944
23	CREVAL S D S BHATTACHARJI J N AND DAS B C	DETERMINATION OF BLOOD CROUPS FROM MEALS OF BLOOD SUCKING INSECTS	*Indian Med Gaz* **79,** 503 July 1944
24	CREVAI S D S AND ROY CHOWDHURY A B	THE Rh HEM(AGGLUTIN)OGEN	*Jour I M A* **14** 45 1944
25	GREVAL S D S	Rh rh Hr AND SOMATIC MANIFESTATION OF A RECESSIVE CHARACTER IN THE PRESENCE OF THE DOMINANT CHARAC TER	*Current Science* No 13 273 Dec 1944
26	CREVAL S D S	THE USE OF BLOOD TESTS IN EXCLUDING PATERNITY AND MATERNITY	*Indian Med Ga* **80,** 204 April 1945
27	CRFVAI S D S AND ROY CHOWDHURY A B	Rh NEGATIVE RATE FOR INDIANS	*Nature* **157** 411 30th March 1946
28	GREVAI S D S ROY CHOWDHURY A B AND BAN NERJI B	INTRA CROUP REACTION IN AN Rh EXPECTANT MOTHER AND ASSOCIATED CONSIDERATIONS	*Jour I M A* **15,** 112 1946
29	GREVAL S D S ROY CHOWDHURY A B AND BAN NERJI B	A PRESUMPTIVE CASE OF ERYTHRO BLASTOSIS FOETALIS DUE TO Rh INCOM PATIBILITY	*Indian Med Gaz* **81,** 243 1946

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